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(54) Title: INHIBITOR OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR

(57) Abstract

The vascular endothelial cell growth factor (VEGF) inhibitors of the present invention are naturally occurring or recombinantly engineered soluble forms with or without a C-terminal transmembrane region of the receptor for VEGF, a very selective growth factor for endothelial cells. The soluble forms of the receptors will bind the growth factor with high affinity but do not result in signal transduction. These soluble forms of the receptor bind VEGF and inhibit its function.

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TITLE OF THE DISCLOSURE

INHIBITOR OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR

BACKGROUND OF THE DISCLOSURE

Recently a new class of cell-derived dimeric mitogens with selectivity for vascular endothelial cells has been identified and designated vascular endothelial cell growth factor (VEGF). VEGF has been purified from conditioned growth media of rat glioma cells [Conn et al., (1990), Proc. Natl. Acad. Sci. U.S.A., 87, pp 2628-2632]; and conditioned growth media of bovine pituitary folliculo stellate cells [Ferrara and Henzel, (1989), Biochem. Biophys. Res. Comm., 161, pp. 851-858; Gozpadorowicz et al., (1989), Proc. Natl. Acad. Sci. U.S.A., 86, pp. 7311-7315] and conditioned growth medium from human U937 cells [Connolly, D. T. et al. (1989), Science, 246, pp. 1309-1312]. VEGF is a dimer with an apparent molecular mass of about 46 kDa with each subunit having an apparent molecular mass of about 23 kDa.

VEGF has some structural similarities to platelet derived growth factor (PDGF), which is a mitogen for connective tissue cells but not mitogenic for vascular endothelial cells from large vessels.

The membrane-bound tyrosine kinase receptor, known as FLT, was shown to be a VEGF receptor [DeVries, C. et al., (1992), Science, 255, pp.989-991]. The FLT receptor specifically binds VEGF which induces mitogenesis. Another form of the VEGF receptor, designated KDR, is also known to bind VEGF and induce mitogenesis. The partial cDNA sequence and nearly full length protein sequence of KDR is known as well [Terman, B.I. et al., (1991) Oncogene 6, pp. 1677-1683; Terman, B.I. et al., (1992) Biochem. Biophys. Res. Comm. 187, pp. 1579-1586].

Persistent angiogenesis may cause or exacerbate certain diseases such as psoriasis, rheumatoid arthritis, hemangiomas, angiofibromas, diabetic retinopathy and neovascular glaucoma. An inhibitor of VEGF activity would be useful as a treatment for such diseases and other VEGF-induced pathological angiogenesis and vascular permeability conditions, such as tumor vascularization.

SUMMARY OF THE DISCLOSURE

A naturally-occurring FLT messenger RNA (mRNA) was identified and cloned from vascular endothelial cells. This mRNA is shown to encode most of the extracellular, or soluble, portion of the VEGF receptor, FLT. Soluble receptor molecules including

forms containing a C-terminal transmembrane region are also recombinantly engineered for this and other VEGF receptors. These soluble receptors, comprising truncated and modified forms are expressed in recombinant host cells and have VEGF binding properties. The soluble receptor proteins are useful as inhibitors of VEGF activity since they will bind available VEGF preventing it from activating its functional receptors on vascular endothelial cells and could form non-functional heterodimers with full-length membrane anchored VEGF receptors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 - A schematic diagram of full length VEGF receptors (FLT and KDR), the soluble VEGF receptors (sVEGF-RI and sVEGF-RII) and the soluble receptors containing the C-terminal transmembrane region (sVEGF-RTMI and sVEGF-RTMII) are shown with the protein domains of each.

Figure 2 - The DNA sequence of the sVEGF-RI soluble VEGF receptor/VEGF inhibitor is shown.

Figure 3 - The amino acid sequence of the sVEGF-RI soluble VEGF receptor/VEGF inhibitor is shown.

Figure 4 - Demonstration that recombinant host cells express sVEGF-RI is shown by

the formation of high molecular weight complexes of sVEGF-RI and $[^{125}I]$VEGF and separated by size exclusion chromatography.

Figure 5 - A 12.5% polyacrylamide electrophoretic gel is shown which demonstrates the high degree of purity obtained for sVEGF-RI.

Figure 6 - Cross-linked products of sVEGF-RI and $[^{125}I]$VEGF are shown at about 145 kDa, and at about 245 kDa.

Figure 7A and 7B - Analysis of VEGF binding to sVEGF-RI (A) and corresponding Scatchard plot (B).

Figure 8 - Inhibition of $[^{125}I]$VEGF binding to HUVECs by sVEGF-RI is demonstrated.

Figure 9 - Inhibition of VEGF-mediated mitogenesis on HUVECs is shown using sVEGF-RI.

Figure 10 - The nucleotide sequence encoding sVEGF-RII is shown.

Figure 11 - The amino acid sequence for sVEGF-RII is shown.

Figure 12 - The nucleotide sequence encoding sVEGF-RTMII is shown.

Figure 13 - The amino acid sequence for sVEGF-RTMII is shown.

Figure 14 - The nucleotide sequence encoding sVEGF-RTMI is shown.

Figure 15 - The amino acid sequence for sVEGF-RTMI is shown.

Figure 16 - A diagram of pmFLT is shown.

Figure 17 - A diagram of pKDRA is shown.

DETAILED DESCRIPTION OF THE DISCLOSURE

The present invention relates to cDNA encoding a soluble VEGF receptor protein (sVEGF-R) which is isolated from VEGF receptor producing cells or is recombinantly engineered from VEGF receptor-encoding DNA. sVEGF-R, as used herein, refers to a protein which can specifically bind to a vascular endothelial cell growth factor without stimulating mitogenesis of vascular endothelial cells.

The amino acid sequence of FLT is known, [Shibuya, M. et al., (1990), Oncogene, 5, pp.519-524] and corresponds to the full length cell-associated VEGF tyrosine kinase receptor. Other VEGF receptors are known to exist. Other known VEGF receptors include,

but are not limited to KDR [Terman (1991), supra., and Terman (1992), supra.]. Mammalian cells capable of producing FLT, KDR and other VEGF receptors include, but are not limited to, vascular endothelial cells. Mammalian cell lines which produce FLT or KDR and other VEGF receptors include, but are not limited to, human endothelial cells. The preferred cells for the present invention include human umbilical vein endothelial cells (HUVEC).

Other cells and cell lines may also be suitable for use to isolate sVEGF-R cDNA. Selection of suitable cells may be done by screening for sVEGF-R binding activity on cell surfaces, in cell extracts or conditioned medium or by screening for gene expression by PCR or hybridization. Methods for detecting soluble receptor activity are well known in the art [Duan, D-S. R. et al., (1991) J.Biol.Chem., 266, pp.413-418] and measure the binding of labelled VEGF. Cells which possess VEGF binding activity in this assay may be suitable for the isolation of sVEGF-R cDNA.

Full length FLT producing cells such as human HUVEC cells (American Type Culture Collection, ATCC CRL 1730) [Hoshi, H. and McKeehan, W.L., Proc. Natl. Acad. Sci. U.S.A., (1984) 81, pp. 6413-6417] are grown according to the recommended culture conditions of the ATCC. Full length FLT, and KDR VEGF receptors as well as extracellular region (sVEGF-RI and sVEGF-RII) and extracellular region plus transmembrane region forms (sVEGF-RTMI and sVEGF-RTMII) are shown in Figure 1. The full length receptor has an extracellular ligand

binding region composed of about seven immunoglobulin-like domains, a membrane spanning sequence (transmembrane domain) and intracellular tyrosine kinase domains. The inhibitory forms of this receptor, which are the subject of the present invention, are also shown in Figure 1 and lack the intracellular kinase domains, and for some inhibitors, the transmembrane sequence and the C-terminal most Ig-like extracellular domain.

Any of a variety of procedures may be used to molecularly clone sVEGF-R cDNA. These methods include, but are not limited to, direct functional expression of the sVEGF-R gene following the construction of an sVEGF-R-containing cDNA library in an appropriate expression vector system.

Another method is to screen a sVEGF-R-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a labelled oligonucleotide probe designed from the predicted amino acid sequence of sVEGF-R. The preferred method consists of screening a sVEGF-R-containing cDNA library constructed in a bacteriophage or plasmid shuttle vector with a partial cDNA encoding at least part of the full length FLT protein. This partial cDNA is obtained by the specific PCR amplification of sVEGF-R DNA fragments through the design of oligonucleotide primers from the known sequence of the full length FLT-encoding DNA.

It is readily apparent to those skilled in the art that other types of libraries, as well as

libraries constructed from other cells or cell types, may be useful for isolating sVEGF-R-encoding DNA. Other types of libraries include, but are not limited to, cDNA libraries derived from other cells or cell lines other than HUVECs and genomic DNA libraries.

It is readily apparent to those skilled in the art that suitable cDNA libraries may be prepared from cells or cell lines which have sVEGF-R activity. The selection of cells or cell lines for use in preparing a cDNA library to isolate sVEGF-R cDNA may be done by first measuring secreted sVEGF-R activity using the VEGF binding assay described fully herein.

Preparation of cDNA libraries can be performed by standard techniques well known in the art. Well known cDNA library construction techniques can be found for example, in Maniatis, T., Fritsch, E.F., Sambrook, J., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982).

It is also readily apparent to those skilled in the art that DNA encoding sVEGF-R may also be isolated from a suitable genomic DNA library. Construction of genomic DNA libraries can be performed by standard techniques well known in the art. Well known genomic DNA library construction techiques can be found in Maniatis, T., Fritsch, E.F., Sambrook, J. in Molecular Cloning: A Laboratory Manuel (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982).

Another means of obtaining sVEGF-R molecules is to recombinantly engineer them from DNA encoding the

partial or complete amino acid sequence of a VEGF receptor. Examples of other VEGF receptors include, but are not limited to, KDR. Using recombinant DNA techniques, DNA molecules are constructed which encode at least a portion of the VEGF receptor capable of binding VEGF without stimulating mitogenesis. Standard recombinant DNA techniques are used such as those found in Maniatis, et al., supra.

Using one of the preferred methods of the present invention, cDNA clones encoding sVEGF-R are isolated in a two-stage approach employing polymerase chain reaction (PCR) based technology and cDNA library screening. In the first stage, DNA oligonucleotides derived from the extracellular domain sequence information from the known full length FLT, KDR or other VEGF receptor is used to design degenerate oligonucleotide primers for the amplification of sVEGF-R-specific DNA fragments. In the second stage, these fragments are cloned to serve as probes for the isolation of complete sVEGF-R cDNA from a commercially available lambda gt10 cDNA library (Clontech) derived from HUVEC cells (ATCC CRL 1730).

These PCR derived products were used as hybridization probes for screening a lambda gt10 cDNA library derived from HUVECs (Clontech). Plating and plaque lifts of the library were performed by standard methods (T. Maniatis, E.F. Fritsch, J. Sambrook, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1982). The probes were random-primed labelled with

^{32}P-dCTP to high specific activity and a separate screening of the library (1×10^6 plaques per screen) was conducted with each probe. The probes were added to hybridization buffer (50% formamide, 5X Denhardts, 6X SSC (1X SSC = 0.15 M NaCl, 0.015 M Na_3citrate$\cdot 2H_2O$, pH 7.0), 0.1% SDS, 100 μg/ml salmon sperm DNA) at 1×10^6 cpm/ml.

Four positively hybridizing phage were detected using the flt-specific probe. These positively hybridizing phage were observed to be less than full length flt.

Two flt cDNA clones of about 2.0 kb and 2.7 kb in length were subcloned into pGEM vectors (Promega) and bi-directionally sequenced in their entirety by the chain termination method (Sanger et al., (1977) P.N.A.S. USA, 74, pp. 5463-5467,) and shown to contain a single open reading frame of about 569 amino acids. Sequence analysis demonstrated that a portion of the 5' flt coding region was missing from these clones. The remainder of the 5' end was cloned using PCR and combined with the DNA of the clones lacking the 5' end to yield a single open reading frame encoding about 687 amino acids.

The sequence for the cDNA encoding flt-derived sVEGF-RI is shown in Table 1, and was identified in clones 7 and 11. The deduced amino acid sequence of sVEGF-RI from the cloned cDNA is shown in Table 2. Inspection of the deduced amino acid sequence reveals the presence of a single, large open reading frame of 687 amino acids. By comparison with amino

acid sequence of the full length FLT VEGF receptor, 31 amino acids are encoded at the C-terminal end of the cDNA which are different from those of FLT.

Using another of the preferred methods of the present invention, DNA encoding sVEGF-R is constructed from a DNA sequence encoding a VEGF receptor. For purposes of illustration, DNA encoding the VEGF receptor known as KDR was utilized. Using the receptor DNA sequence, a DNA molecule is constructed which encodes the extracellular domain of the receptor, or the VEGF binding domain only and is denoted sVEGF-RII. Restriction endonuclease cleavage sites are identified within the receptor DNA and can be utilized directly to excise the extracellular-encoding portion. In addition, PCR techniques as described above may be utilized to produce the desired portion of DNA. It is readily apparent to those skilled in the art that other techniques, which are standard in the art, may be utilized to produce sVEGF-R molecules in a manner analagous to those described above. Such techniques are found, for example, in Maniatis et al., supra.

Additional truncated forms of the VEGF receptor are constructed which contain the transmembrane region. Retention of the transmembrane may facilitate orientation of the inhibitor molecule at the target cell surface. Examples of transmembrane region containing inhibitor molecules include but are not limited to those shown in Figure 1. sVEGF-RTMI and sVEGF-RTMII, as shown in Figure 1, are FLT-related and KDR-related, respectively, transmembrane region

containing receptor inhibitors. Construction of transmembrane region containing molecules, such as sVEGF-RTMI and sVEGF-RTMII, is done by standard techniques known in the art including but not limited to utilizing convenient restriction endonuclease cleavage sites or PCR techniques as described herein. It is readily understood by those skilled in the art that various forms of the inhibitors of a VEGF receptor, as disclosed herein, containing only the extracellular region or containing, in addition, the transmembrane region may be constructed which have substantially the same activity.

The cloned sVEGF-R cDNA obtained through the methods described above may be recombinantly expressed by molecular cloning into an expression vector containing a suitable promoter and other appropriate transcription regulatory elements, and transferred into prokaryotic or eukaryotic host cells to produce recombinant sVEGF-R. Techniques for such manipulations are fully described in Maniatis, T, et al., supra, and are well known in the art.

Expression vectors are defined herein as DNA sequences that are required for the transcription of cloned copies of genes and the translation of their mRNAs in an appropriate host. Such vectors can be used to express eukaryotic genes in a variety of hosts such as bacteria, bluegreen algae, fungal cells, yeast cells, plant cells, insect cells and animal cells.

Specifically designed vectors allow the shuttling of DNA between hosts such as bacteria-yeast

or bacteria-animal or bacteria-insect cells. An appropriately constructed expression vector should contain: an origin of replication for autonomous replication in host cells, selectable markers, a limited number of useful restriction enzyme sites, a potential for high copy number, and active promoters. A promoter is defined as a DNA sequence that directs RNA polymerase to bind to DNA and initiate RNA synthesis. A strong promoter is one which causes mRNAs to be initiated at high frequency. Expression vectors may include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids or viruses.

A variety of mammalian expression vectors may be used to express recombinant sVEGF-R in mammalian cells. Commercially available mammalian expression vectors which may be suitable for recombinant sVEGF-R expression, include but are not limited to, pMClneo (Stratagene), pXTl (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and gZD35 (ATCC 37565).

DNA encoding sVEGF-R may also be cloned into an expression vector for expression in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria, yeast, mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to

drosophila, moth, mosquito and armyworm derived cell lines. Cell lines derived from mammalian species which may be suitable and which are commercially available, include but are not limited to, CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171). Insect cell lines which may be suitable and are commercially available include but are not limited to 3M-S (ATCC CRL 8851) moth (ATCC CCL 80) mosquito (ATCC CCL 194 and 195; ATCC CRL 1660 and 1591) and armyworm (Sf9, ATCC CRL 1711).

The expression vector may be introduced into host cells via any one of a number of techniques including but not limited to transformation, transfection, liposome or protoplast fusion, and electroporation. The expression vector-containing cells are clonally propagated and individually analyzed to determine whether they produce sVEGF-R protein. Identification of sVEGF-R expressing host cell clones may be done by several means, including but not limited to immunological reactivity with anti-sVEGF-R antibodies, binding to radiolabelled VEGF, and the presence of host cell-secreted sVEGF-R activity.

Expression of sVEGF-R DNA may also be performed using in vitro produced synthetic mRNA. Synthetic mRNA can be efficiently translated in various cell-free systems, including but not limited to wheat germ extracts and reticulocyte extracts, as well as

efficiently translated in cell based systems, including but not limited to microinjection into frog oocytes, with microinjection into frog oocytes being preferred.

Levels of sVEGF-R protein produced by host cells may be quantitated by immunoaffinity and/or ligand affinity techniques. sVEGF-R-specific affinity beads or sVEGF-R-specific antibodies are used to isolate ^{35}S-methionine labelled or unlabelled sVEGF-R protein. Labelled sVEGF-R protein is analyzed by SDS-PAGE. Unlabelled sVEGF-R protein is detected by Western blotting, ELISA or RIA assays employing sVEGF-R specific antibodies, or by ligand blotting with labelled VEGF.

Following expression of sVEGF-R in a recombinant host cell, sVEGF-R protein may be recovered to provide sVEGF-R in active form, capable of binding VEGF without stimulating mitogenesis. Several sVEGF-R purification procedures are available and suitable for use. sVEGF-R may be purified from cell lysates and extracts, or from conditioned culture medium, by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography, reversed phase chromatography, heparin sepharose chromatography, VEGF ligand affinity chromatography, and hydrophobic interaction chromatography.

In addition, recombinant sVEGF-R can be separated from other cellular proteins by use of an immuno-affinity column made with monoclonal or

polyclonal antibodies specific for full length sVEGF-R, or polypeptide fragments of sVEGF-R.

<u>Identification of sVEGF-RI</u> - In an attempt to clone the VEGF receptor cDNA (flt) a HUVEC λgt10 cDNA library was screened with a DNA probe derived from the extracellular domain of the membrane bound or full length form of this receptor as shown in Figure 1. Four incomplete clones, all lacking various lengths of 5' coding sequence, were isolated from screening a total of 1 x 10^6 plaques. Two of these isolates represent partial clones that were identical to full length flt, one of which contained the complete 3' coding region of the form described by Shibuya <u>et al</u>., supra. The other two clones were identical to full length flt up to base pair number 2219 (Table 1 and Figure 2) where they then diverged from full length flt. These clones (clone 7 and 11) coded for an additional unique 31 amino acids before the open reading frame is terminated by a TAA codon (Table 2 and Figure 3).

Clone 7 and 11 coded for a protein with a predicted molecular mass of about 75 kDa containing 12 putative N-linked glycosylation sites. This version of the receptor was missing the transmembrane and intracellular kinase domains and thus coded for a natural soluble form of the VEGF receptor (sVEGF-RI). Further, the protein molecule predicted by sVEGF-RI has only the first six Ig-like domains, missing the one closest to the transmembrane sequence (Figure 1). The

31 amino acids at the C-terminal end of sVEGF-RI contain two cysteine residues, but does not resemble an Ig domain.

Expression of sVEGF-RI in Sf9 cells - To analyze the binding and biological properties of this form of the receptor, the protein was expressed using a baculovirus expression system. Clone 7 was missing about 350 base pairs of coding sequence at the 5' end. This region was cloned by PCR using the primers described above and in Example 1. A clone containing the complete coding region of sVEGF-RI was constructed by combining the 5' PCR fragment with sVEGF-RI clone 7 which overlapped at a SacI site. The 5' EcoRI site was then changed to a BamHI site and the full length sVEGF-RI was cloned into pBluebac III (Invitrogen) as a BamHI/BamHI fragment. A recombinant baculovirus P-3 stock containing the sVEGF-RI gene 3' in relation to the polyhedrin promoter was then prepared as described herein.

Culture media from small scale infections were tested for the ability to form high molecular weight complexes with [^{125}I]VEGF. The labeled ligand and culture media from the baculovirus infected cells were combined and incubated. The reactions were then analyzed by size exclusion chromatography. When the wild-type infected culture medium was mixed with the radioactive ligand (Figure 4) a single radioactive peak was observed. However, when the sVEGF-RI infected culture medium was used, a high molecular weight complex was formed, as evident by the appearance of a

second peak in this reaction eluting near the void volume of the column. This experiment showed that the natural soluble form of the FLT VEGF receptor, sVEGF-RI, forms a high molecular weight complex with VEGF.

The recombinantly produced sVEGF-R is purified from the recombinant host cell extracts or cell culture fluid using heparin-sepharose column chromatography which specifically binds the sVEGF-R protein. The heparin-sepharose bound VEGF-R column is washed using a suitable buffer containing between 0.1M and 0.6M NaCl which removes contaminating proteins without significant loss of sVEGF-R. The sVEGF-R is eluted from the heparin-sepharose column using a suitable buffer containing about 1M NaCl, yielding substantially purified sVEGF-R.

<u>Binding of the sVEGF-RI to VEGF</u> - The binding of ^{125}I-labelled VEGF to sVEGF-RI was characterized by crosslinking, and by complex formation with sVEGF-RI absorbed to 96 well plates.

The crosslinked products are shown in Figure 6. The sVEGF-RI was cross-linked to [^{125}I]VEGF (lane 1); in the presence of unlabelled VEGF (lane 2) and unlabelled bFGF (lane 3). Two high molecular weight bands (about 145 kDa and 245 kDa) were formed in the sVEGF-RI and [^{125}I]VEGF containing reaction, and in the sVEGF-RI and [^{125}I]VEGF plus an excess of unlabelled bFGF reaction. The two high molecular weight bands were not present when sVEGF-RI was

incubated with [^{125}I]VEGF plus an excess of unlabelled VEGF, demonstrating the specificity of sVEGF-RI for VEGF, and the ability of sVEGF-RI to form a dimer. The 145 kDa band is presumably a crosslinked complex containing one receptor molecule (about 100 kDa) and a VEGF dimer (about 46 kDa). As shown in Figure 6 complexes containing two receptor molecules (about 245 kDA) were also observed. This suggests that each VEGF dimer can bind one or two receptor molecules and that the soluble form of the VEGF receptor may undergo ligand-induced dimerization.

The affinity of sVEGF-RI for VEGF was evaluated by absorbing sVEGF-RI to the surface of a 96 well plate, followed by blocking the nonspecific sites with 0.5% gelatin. Variable amounts of labeled ligand were added to each well. These results demonstrate that sVEGF-RI binds VEGF with high affinity with an apparent K_d of about 20pM (Figure 7). Since the soluble form of the receptor is missing the Ig domain closest to the transmembrane spanning region, this domain is not required for ligand binding.

The sVEGF-RI is shown to inhibit binding of VEGF to HUVECs by incubating cultured HUVECs with [^{125}I]VEGF and various amounts of sVEGF-RI. Following incubation, the cells are washed to remove unbound [^{125}I]VEGF. The cells are then solubilized and the amount of cell-associated ^{125}I is determined by gamma counter, which demonstrates the amount of [^{125}I]VEGF which was capable of binding to the cellular VEGF receptor in the presence of sVEGF-RI. Using this

method, it is demonstrated that sVEGF-RI was capable of inhibiting [^{125}I]VEGF binding to HUVECs VEGF receptor (see Figure 8).

Since sVEGF-RI was able to inhibit VEGF binding to cell receptors, it was then determined that sVEGF-RI could inhibit VEGF induced mitogenesis. Cells are preincubated with sVEGF-RI and then incubated with VEGF in the presence of [^{3}H]thymidine. Following incubation, the amount of cellular DNA-incorporated [^{3}H]thymidine is measured which indicates whether VEGF has induced mitogenesis and caused [^{3}H]thymidine to be incorporated into cellular DNA. The presence of sVEGF-RI inhibits the ability of VEGF to stimulate mitogenesis as shown in Figure 9.

The inhibitor of the present invention can be used for the inhibition of VEGF activity. The inhibitor can be used either topically or intravascularly. For topical applications the formulation would be applied directly at a rate of about 10 ng to about 1 mg/cm^2/day. For intravaneous applications, the inhibitor is used at a rate of about 1 µg to about 10 mg/kg/day of body weight. For internal use, the formulation may be released directly into the region to be treated either from implanted slow release polymeric material or from slow release pumps or repeated injections. The release rate in either case is about 100 ng to about 100 µg/day/cm^3.

For non-topical application the VEGF inhibitor is administered in combination with pharmaceutically acceptable carriers or diluents such

as phosphate buffer, saline, phosphate buffered saline, Ringer's solution, and the like, in a pharmaceutical composition, according to standard pharmaceutical practice. For topical application, various pharmaceutical formulations are useful for the administration of the active compound of this invention. Such formulations include, but are not limited to, the following: ointments such as hydrophilic petrolatum or polyethylene glycol ointment; pastes which may contain gums such as xanthan gum; solutions such as alcoholic or aqueous solutions; gels such as aluminum hydroxide or sodium alginate gels; albumins such as human or animal albumins; collagens such as human or animal collagens; celluloses such as alkyl celluloses, hydroxy alkyl celluloses and alkylhydroxyalkyl celluloses, for example methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; polyoxamers such as Pluronic® Polyols exemplified by Pluronic® F-127; tetronics such as tetronic 1508; and alginates such as sodium alginate.

The following examples are provided as illustrative of the present invention without, however, limiting the same thereto.

EXAMPLE 1

Cloning flt-related sVEGF-RI - A 580 base pair DNA probe for flt was obtained by PCR of the HUVEC phage library using the primers 5' GCACCTTGGTTGTGGCTGAC 3'

(SEQ. ID. No.: 1) and 5' TGGAATTCGTGCTGCTTCCTGGTCC 3'(SEQ. ID. No.: 2). The resulting DNA fragment was cloned into pGEM3Z as a XbaI/EcoRI fragment. The probe was prepared by the random priming method [Feinberg, A.P. and Vogelstein, B., (1983) Anal.Biochem., 132, pp.6-13] using the megaprime kit (Amersham) at a specific activity of 1 X 10^7 cpm/ng. The HUVEC cDNA library was plated at a density of 5 X 10^4 plaques/150 cm plate then about 1 X 10^6 plaques were screened by hybridization as previously described [Maniatis, T. et al., supra]. Briefly, following prehybridization at 42°C for 2 hours in 50% formamide, 5X SSC, 5X Denhardt's solution, 0.1% SDS, 100 µg/ml salmon sperm DNA (hybridization buffer) the filters were hybridized with the probe for 16 hours at 42°C in hybridization buffer. The filters were washed one time for 15 min at room temperature in 2X SSC then three times at 55°C in 0.1 X SSC. Four positive plaques were identified and rescreened two additional times to obtain homogeneous isolates. Inserts were cloned into pGEM3Z for DNA sequence analysis. Two of these clones were identified which contained less than the full length flt coding region. DNA sequence analysis showed that these clones lacked the 5' coding region of flt. The DNA sequence is shown in Table 1 and Figure 2, and the deduced amino acid sequence is shown in Table 2 and Figure 3. The 5' end of flt was cloned by PCR using the primers 5' GGAATTCCGCGCTCACCATGGTCAGC 3' (SEQ.ID.NO.:3) and 5' TTTGAATTCACCCGGCAGGGAATGACG 3' (SEQ.ID.NO.:4). The PCR fragment generated with this set of primers was cloned into flt clone 7 as an EcoRI/SacI fragment.

TABLE 1

GCGGACACTCCTCTCGGCTCCTCCCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGG

CTCGGGTGCAGCGGCCAGCGGGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTC

CTGGCTGGAGCCGCGAGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCGAACGAGAGG

ACGGACTCTGGCGGCCGGGTCGTTGGCCGGGGGAGCGCGGGCACCGGGCGAGCAGGCCG

CGTCGCGCTCACC ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG

TGC GCG CTG CTC AGC TGT CTG CTT CTC ACA GGA TCT AGT TCA GGT

TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG CAC

ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA

GCA GCC CAT AAA TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC

GAA AGG CTG AGC ATA ACT AAA TCT GCC TGT GGA AGA AAT GGC AAA

CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC CAC

ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG

AAG AAG GAA ACA GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA

GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT ATA

CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG

TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT

TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC

TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC

TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC

ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA

CCA CGC CCA GTC AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT
TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC TGG
AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA
ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT
ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT
CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG
CAT ATA TAT GAT AAA GCA TTC ATC ACT GTG AAA CAT CGA AAA CAG
CAG GTG CTT GAA ACC GTA GCT GGC AAG CGG TCT TAC CGG CTC TCT
ATG AAA GTG AAG GCA TTT CCC TCG CCG GAA GTT GTA TGG TTA AAA
GAT GGG TTA CCT GCG ACT GAG AAA TCT GCT CGC TAT TTG ACT CGT

GGC TAC TCG TTA ATT ATC AAG GAC GTA ACT GAA GAG GAT GCA GGG
AAT TAT ACA ATC TTG CTG AGC ATA AAA CAG TCA AAT GTG TTT AAA
AAC CTC ACT GCC ACT CTA ATT GTC AAT GTG AAA CCC CAG ATT TAC
GAA AAG GCC GTG TCA TCG TTT CCA GAC CCG GCT CTC TAC CCA CTG
GGC AGC AGA CAA ATC CTG ACT TGT ACC GCA TAT GGT ATC CCT CAA
CCT ACA ATC AAG TGG TTC TGG CAC CCC TGT AAC CAT AAT CAT TCC
GAA GCA AGG TGT GAC TTT TGT TCC AAT AAT GAA GAG TCC TTT ATC
CTG GAT GCT GAC AGC AAC ATG GGA AAC AGA ATT GAG AGC ATC ACT
CAG CGC ATG GCA ATA ATA GAA GGA AAG AAT AAG ATG GCT AGC ACC
TTG GTT GTG GCT GAC TCT AGA ATT TCT GGA ATC TAC ATT TGC ATA

GCT TCC AAT AAA GTT GGG ACT GTG GGA AGA AAC ATA AGC TTT TAT

ATC ACA GAT GTG CCA AAT GGG TTT CAT GTT AAC TTG GAA AAA ATG

CCG ACG GAA GGA GAG GAC CTG AAA CTG TCT TGC ACA GTT AAC AAG

TTC TTA TAC AGA GAC GTT ACT TGG ATT TTA CTG CGG ACA GTT AAT

AAC AGA ACA ATG CAC TAC AGT ATT AGC AAG CAA AAA ATG GCC ATC

ACT AAG GAG CAC TCC ATC ACT CTT AAT CTT ACC ATC ATG AAT GTT

TCC CTG CAA GAT TCA GGC ACC TAT GCC TGC AGA GCC AGG AAT GTA

TAC ACA GGG GAA GAA ATC CTC CAG AAG AAA GAA ATT ACA ATC AGA

GGT GAG CAC TGC AAC AAA AAG GCT GTT TTC TCT CGG ATC TCC AAA

TTT AAA AGC ACA AGG AAT GAT TGT ACC ACA CAA AGT AAT GTA AAA

CAT TAA

AGGACTCATTAAAAAGTAACAGTTGTCTCATATCATCTTGATTTATTGTCACTGTTG

CTAACTTTCAGGCTCGGAGGAGATGCTCCTCCCAAAATGAGTTCGGAGATGATAGCA

GTAATAATGAGACCCCCGGGCTCCAGCTCTGGGCCCCCCATTCAGGCCGAGGGGGCT

GCTCCGGGGGGCCGACTTGGTGCACGTTTGGATTTGGAGGATCCCTGCACTGCCTTC

TCTGTGTTTGTTGCTCTTGCTGTTTTCTCCTGCCTGATAAACAACAACTTGGGATGA

TCCTTTCCATTTTGATGCCAACCTCTTTTTATTTTTAAGCGGCGCCCTATAGT
(SEQ. ID. NO.: 5)

TABLE 2

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu

Cys Ala Leu Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly

Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His

Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu

Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser

Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys

Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His

Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys

Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile

His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr
Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly
Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr
Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu
Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr
Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn
Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp
Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg
Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu

Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys

Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val

His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln

Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser

Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys

Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg

Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly

Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr

Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu

Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln

Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser

Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile

Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr

Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr

Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile

Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr

Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met

Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys

Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn

Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile

Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val

Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val

Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg

Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys

Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys

His ••• (SEQ. ID. NO.: 6)

EXAMPLE 2

Expression of sVEGF-RI in Sf9 insect cells - The full length sequence encoding sVEGF-RI was cloned as an EcoRI/BamHI fragment into pGEM3Z. The EcoRI site was then modified to a BamHI site and cloned into pBlueBac III 3' of the polyhedrin promoter (psFLTblue). This plasmid was transfected into Sf9 armyworm cells using liposomes. After 48 hours the medium from the transfected cells which contains recombinant polyhedrin virus particles, was harvested. Dilutions (10^3 - 10^4 fold) of the virus were prepared and plaque purified in soft agar containing 150 µg/ml 5-bromo-4-chloro-3-

indolyl-ß-D-galactoside. Recombinant plaques were identified by blue color and used to infect Sf9 cells (5×10^5 cells/well) in 12 well plates. Medium (100 µl) from polyhedrin minus infections was used to prepare P-2 viral stocks by infecting 2.5×10^6 cells in a T-25 flask. Large scale high titer P-3 viral stocks were then prepared by infecting Sf9 cells (500 ml at 2×10^6 cells/ml) with 5 ml of the P-2 stock then incubating at 27°C for 5 - 6 days and the medium was harvested by centrifugation. Protein expression was accomplished by infecting cells at a density of 2- 2.5 $\times 10^6$ cells/ml with a multiplicity of infection of 5 - 10. Twenty four hours after infection the cells were changed to a serum free medium (SF900II, Gibco BRL), incubated for an additional 48 hours and the medium was collected. This conditioned medium contains the recombinantly expressed sVEGF-RI protein.

EXAMPLE 3

Iodination of VEGF - ^{125}I-labeled human recombinant VEGF was prepared by the chloramine T method (Hunter, W.M. and Greenwood, F.C., (1962) Nature (London), 194, pp. 495-496). Briefly, 1 µg of VEGF in 30% acetonitrile/0.1% trifluroacetic acid was adjusted to pH 7.1 by the addition of 1/3 volume of 0.4 M sodium phosphate buffer, pH 7.1. Freshly dissolved chloramine T (4 µl of a 2 mg/ml stock in 0.1 M sodium phosphate buffer, pH 7.1) was added to the VEGF solution and reacted for 45 seconds at room temperature (total

volume of 150 µl). The reaction was stopped by the addition of 50 µl of 10 mM KI and 50 µl of 2 mg/ml meta bisufite. The labeled ligand was separated from the free ^{125}I by gel filtration on a 0.7 X 15 cm Sephadex G-25 column equilibrated in PBS with 1 mg/ml gelatin. Fractions were counted in a Packard γ counter, aliquoted and stored at -70°C. VEGF was labeled to a specific activity of 5×10^5 to 1×10^6 cpm/ng.

<u>Gel Filtration Chromatography</u> - Receptor-ligand complex was formed by incubating 10 µl of ^{125}I-labeled VEGF (10^5 cpm) with 100 µl of either wild-type or baculovirus sVEGF-RI-containing, infected Sf9 cell culture medium overnight at room temperature. The reaction products were separated on a Sephacryl S200 gel filtration column (0.7 X 25 cm) equilibrated in PBS, 1 mg/ml gelatin, at a flow rate of 15 ml/hr. Fractions (0.75 ml) were collected and analyzed in a γ counter. Receptor-ligand complexes pass quickly through the column while the free labelled VEGF passes through more slowly. The results of this experiment shown in Figure 4 demonstrate the formation of a high molecular weight complex between labelled VEGF and sVEGF-RI protein. This shows that sVEGF-RI binds VEGF.

<u>Crosslinking</u> - Purified sVEGF-RI (1-10ng) was added to 25 µl of binding buffer (Dulbecco's Modified Eagle's medium (DME), 25 mM HEPES, pH 7.5, 0.3% gelatin), and 1×10^5 cpm of [^{125}I]-VEGF was added (Figure 6, lane 1) with either 200ng of unlabelled VEGF (lane 2) or bFGF

(lane 3), then incubated 2 to 16 hours at room temperature. Bis(sulfosuccinimidyl)suberate (Pierce) crosslinker was added to a final concentration of 1 mM. The reaction was stopped after 15 min by the addition of boiling SDS PAGE sample buffer. The crosslinked products were separated by SDS PAGE on a 7.5% acrylamide gel and analyzed either by autoradiography or a phosphoimager. The results are shown in Figure 6 and demonstrate that sVEGF-RI binds labelled VEGF by the appearance of two bands of about 145 kDa and 245 kDa. The 145 kDa band consists of one sVEGF-RI molecule and one VEGF molecule (Monomer, M.). The 245 kDa band apparently consists of two sVEGF-RI molecules and one VEGF dimer (D). Free VEGF ligand (L) dimers migrated at about 45 kDA.

Binding assay - The binding of sVEGF-RI to VEGF was analyzed using a 96 well plate assay as described by Duan, D-S. R. et al., supra. Briefly, sVEGF-RI, 50 to 200 µl partially purified by Mono Q chromatography (Pharmacia), was diluted to 10 ml in 25 mM TRIS, pH 7.4, 100 mM NaCl, 20 mM NH_4HCO_3. Aliquots (100 µl) were absorbed to the surface of a 96 well plate for 18 hours at 4°C, the plates were then washed twice with blocking buffer (DME, 25 mM HEPES, pH 7.5, 0.5% gelatin) and the nonspecific sites were blocked in the same buffer for 6 hours at 4°C. The plate was then washed twice in binding buffer. Various amounts of $[^{125}I]$VEGF were added to the wells in a final volume of 100 µl/well and incubated for 2 hours at room

temperature. The wells were washed three times with 100 µl of binding buffer, the bound protein was solubilized with 100 µl of 1% SDS, 0.5% BSA and counted in a γ counter. The results, shown in Figure 7, were analyzed by the method of Scatchard [Scatchard, G., (1949) Ann. N.Y. Acad. Sci., 51, pp. 660-672]. The analysis demonstrates that sVEGF-RI retains high affinity binding for VEGF with a K_d value of about 20 pM. This clearly demonstrates that sVEGF-RI, lacking the transmembrane region and adjacent Ig-like domain, binds VEGF with high affinity and that these regions are not required for VEGF binding.

EXAMPLE 4

Inhibition of VEGF binding by sVEGF-RI - The ability of sVEGF-RI to inhibit VEGF binding to HUVECs was tested. HUVECs were plated at 50,000 cells/well in 24 well plates precoated with gelatin, and allowed to grow to confluence. A constant amount of [^{125}I]VEGF (100,000 cpm) was mixed with various amounts of partially purified sVEGF-RI in binding buffer, in a total volume of 200 µl and preincubated at room temperature for 1 hour. Samples were added to the cells and incubated for 4 hours at 4°C with shaking. The medium was then aspirated and the cells were washed three times with binding buffer. The bound radioactivity was solubilized with 50 mM TRIS-HCl, pH 8.0, 150 mM NaCl, 1% NP40, 1% BSA and counted in a γ counter.

The results are shown in Figure 8. At the highest concentration of sVEGF-RI, VEGF binding to HUVECs was reduced by 70%. It may, however, be difficult to completely inhibit binding to the cellular membrane bound receptor since one molecule of sVEGF-R bound to a VEGF dimer may be able to bind to cell associated receptor to form an inactive (sVEGF-RI)-VEGF-(membrane spanning VEGF receptor) complex.

EXAMPLE 5

Inhibition of VEGF mediated mitogenesis by sVEGF-RI

Mitogenic inhibition - Since sVEGF-RI was able to inhibit VEGF binding to endothelial cells, it was then determined that the soluble receptor could inhibit VEGF induced mitogenesis in HUVECs. HUVECs were plated in gelatin coated 96 well plates at a density of 4000 cells/well in 100 µl of DME supplemented with 10% heat inactivated fetal calf serum plus antibiotics (penicillin G, 100 units/ml; streptomycin sulfate, 100 µg/ml). After 16 hours the medium was changed and test samples were added, cells were preincubated with a variable amount of purified sVEGF-RI for 15 minutes at 37°C before growth factor (10 ng/ml) was added. The cells were incubated for 24 hours then [methyl-^{3}H]thymidine (0.8 µCi/well; 20 Ci/mmol: 1Ci = 37 GBq, final specific activity of 0.8 µCi/nmole) was added followed by incubated for an additional 72 hours at 37°C under 5% CO_2. The cells were then washed twice with Hank's balanced salt solution adjusted to pH 7.5

with 25 mM Hepes, 0.1% BSA. The cells were then lysed, the DNA was solubilized with 0.2 M Na_2CO_3, 0.1 M NaOH, and [^{3}H]thymidine incorporation was quantified by scintillation counting. The results are shown in Figure 9. sVEGF-RI was able to completely inhibit VEGF induced [^{3}H]thymidine incorporation in HUVECs.

EXAMPLE 6

Purification of baculovirus expressed sVEGF-RI from Sf9 cells - Culture medium from Sf9 cells infected with a baculovirus construct designed to express sVEGF-RI (Example 2) was chromatographed through a heparin Sepharose CL-6B (Pharmacia) column (0.7 X 4 cm). The column was washed with 5 volumes of 10 mM Na-phosphate buffer, pH 6.2, 0.1 M NaCl, followed by 6 ml of 10 mM Na-phosphate buffer, pH 6.2, 0.6 M NaCl. The sVEGF-RI was eluted with 10 mM Na-phosphate buffer, pH 6.2, 1.0 M NaCl. Polyacrylamide gel electrophoresis was performed which demonstrated greater than 90% purity (as judged by coomassie blue staining) of the recombinantly produced sVEGF-R (Figure 5). The identity of the protein was confirmed by N-terminal protein sequence analysis. The actual N-terminus (Ser Lys Leu ...) of the recombinant protein differs by two amino acids from that predicted by Shibuya et al., supra. (Ser-Ser-Ser...). The peptidase cleavage site in sVEGF-RI produced in Sf9 cells was between residues gly-26 and ser-27.

EXAMPLE 7

Construction of KDR-related sVEGF-R - Soluble forms of KDR (a known VEGF receptor) [Terman, B.I. et al., (1991) Oncogene 6, pp. 1677-1683; Terman, B.I. et al., (1992) Biochem. Biophys. Res. Comm. 187, pp. 1579-1586] may exist naturally but have not yet been identified. A soluble form of KDR is recombinantly constructed by modifying its coding sequence by PCR using the primers 1) 5' TTTTGGATCCCTGCAGACAGATCTACGTTTGAGAACC 3' (SEQ. ID. NO.: 7) and 2) 5' TTTTGGATCCTTAACGCTCTAGGACTGTGAGC 3' (SEQ. ID. NO.: 8), and pKDRA (the Xhol/EcoRl fragment coding for the extracellular and transmembrane domain of KDR cloned into the EcoRI site of pGEM 7Z obtained from Promega) as a template (Figure 17). This generated a translation stop codon after amino acid residue number 663 of KDR which corresponds to the extracellular domain of full length KDR. This modified fragment is then used to replace the Pstl/BamHl fragment of pKDRA generating a truncated form of the KDR gene (Figure 10) which codes for a soluble receptor denoted sVEGF-RII (Figure 11). The Xhol site at base pair number 257 is then changed to a BamHl site by standard cloning techniques. Another truncated form of the KDR receptor is created with primer 1 shown above, and primer 3) 5' TTTTGGATCCAACGGTCCCTAGGATGATGAC 3' , (SEQ. ID. NO.: 9) (Figure 12). This form of KDR, denoted sVEGF-RTMII, is truncated at the C-terminal side of the transmembrane domain and therefore retains the transmembrane region (Figure 13). A similar form of the FLT receptor is generated by PCR using the

primers 4) 5' AGCACCTTGGTTGTGGCTGACTC 3' (SEQ. ID. NO.: 10) and 5) 5' TTTTGGATCCTTAGATAAGGAGGGTTAATAGG 3' (SEQ. ID. NO.: 11) and plasmid pmFLT (full length flt cloned into the EcoRI site of pGEM3Z obtained from Promega) as a template (Figure 16). The 780 base pair PCR fragment can then be cloned together with the EcoR1/Xba1 fragment from pmFLT to produce an EcoR1/BAMH1 fragment (Figure 14) encoding a truncated form of FLT (denoted sVEGF-RTMI) which retains the transmembrane domain but lacks the cytoplasmic domain (Figure 15). The EcoR1 site at the 5' end of the gene is then modified to a BamH1 site. The resulting truncated forms of KDR and FLT are then cloned into pBluebac111 (Stratagene) for expression in Sf9 insect cells. Characterization of these constructed truncated forms of VEGF receptors is accomplished by the techniques used to characterize sVEGF-RI as in Examples 2, 3, 4, 5, and 6.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Thomas, Kenneth A.
Kendall, Richard L.

(ii) TITLE OF INVENTION: INHIBITOR OF VASCULAR ENDOTHELIAL CELL GROWTH FACTOR

(iii) NUMBER OF SEQUENCES: 18

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Merck & Co., Inc.
(B) STREET: P.O. Box 2000 126 E Lincoln Avenue
(C) CITY: Rahway
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(F) ZIP: 07065-0907

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Wallen, John W.III

(B) REGISTRATION NUMBER: 35,403

(C) REFERENCE/DOCKET NUMBER: 18888

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (908) 594-3905

(B) TELEFAX: (908) 594-4720

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCACCTTGGT TGTGGCTGAC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGGAATTCGT GCTGCTTCCT GGTCC 25

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGAATTCCGC GCTCACCATG GTCAGC 26

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTTGAATTCA CCCGGCAGGG AATGACG 27

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2313 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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GGCTGGAGCC GCGAGACGGG CGCTCAGGGC GCGGGGCCGG CGGCGGCGAA CGAGAGGACG 180

GACTCTGGCG GCCGGGTCGT TGGCCGGGGG AGCGCGGGCA CCGGGCGAGC AGGCCGCGTC 240

GCGCTCACCA TGGTCAGCTA CTGGGACACC GGGGTCCTGC TGTGCGCGCT GCTCAGCTGT 300

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AAATCTGCCT GTGGAAGAAA TGGCAAACAA TTCTGCAGTA CTTTAACCTT GAACACAGCT 540

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AAGGAAACAG AATCTGCAAT CTATATATTT ATTAGTGATA CAGGTAGACC TTTCGTAGAG 660

ATGTACAGTG AAATCCCCGA AATTATACAC ATGACTGAAG GAAGGGAGCT CGTCATTCCC 720

TGCCGGGTTA CGTCACCTAA CATCACTGTT ACTTTAAAAA AGTTTCCACT TGACACTTTG 780

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TCCAATAAAG TTGGGACTGT GGGAAGAAAC ATAAGCTTTT ATATCACAGA TGTGCCAAAT 1920

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GCCAGGAATG TATACACAGG GGAAGAAATC CTCCAGAAGA AAGAAATTAC AATCAGAGGT 2220

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GATTGTACCA CACAAAGTAA TGTAAAACAT TAA 2313

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 687 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
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Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
            20                  25                  30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
        35                  40                  45

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
    50                  55                  60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65                  70                  75                  80
```

Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
85 90 95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
100 105 110

Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
115 120 125

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
130 135 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145 150 155 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
165 170 175

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
180 185 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
195 200 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
210 215 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225 230 235 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
245 250 255

Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
260 265 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
275 280 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
290 295 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305 310 315 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
325 330 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
340 345 350

Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
355 360 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
370 375 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385 390 395 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
405 410 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
420 425 430

Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
435 440 445

Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
450 455 460

Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465 470 475 480

Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
485 490 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
500 505 510

Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
515 520 525

Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
530 535 540

Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545 550 555 560

Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
565 570 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
580 585 590

Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
595 600 605

Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
610 615 620

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625 630 635 640

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
645 650 655

Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys Phe
660 665 670

Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys His
675 680 685

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TTTTGGATCC CTGCAGACAG ATCTACGTTT GAGAAC 36

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTTGGATCC TTAACGCTCT AGGACTGTGA GC 32

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTTGGATCC AACGGTCCCT AGGATGATGA C 31

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGCACCTTGG TTGTGGCTGA CTC 23

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TTTTGGATCC TTAGATAAGG AGGGTTAATA GG 32

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 661 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His Ile
1 5 10 15

Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu Ala Ala
20 25 30

His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser Glu Arg Leu
35 40 45

Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser
50 55 60

Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His Thr Gly Phe Tyr Ser
65 70 75 80

Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser
85 90 95

Ala Ile Tyr Ile Phe Ile Ser Asp Thr Gly Arg Pro Phe Val Glu Met
100 105 110

Tyr Ser Glu Ile Pro Glu Ile Ile His Met Thr Glu Gly Arg Glu Leu
115 120 125

Val Ile Pro Cys Arg Val Thr Ser Pro Asn Ile Thr Val Thr Leu Lys
130 135 140

Lys Phe Pro Leu Asp Thr Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp
145 150 155 160

Asp Ser Arg Lys Gly Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile
165 170 175

Gly Leu Leu Thr Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr
180 185 190

Asn Tyr Leu Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile
195 200 205

Ser Thr Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu
210 215 220

Asn Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp
225 230 235 240

Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg Ile
245 250 255

Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile
260 265 270

Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg
275 280 285

Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val His Ile Tyr Asp
290 295 300

Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln Gln Val Leu Glu Thr
305 310 315 320

Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser Met Lys Val Lys Ala Phe
325 330 335

Pro Ser Pro Glu Val Val Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu
340 345 350

Lys Ser Ala Arg Tyr Leu Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp
355 360 365

Val Thr Glu Glu Asp Ala Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys
370 375 380

Gln Ser Asn Val Phe Lys Asn Leu Thr Ala Thr Leu Ile Val Asn Val
385 390 395 400

Lys Pro Gln Ile Tyr Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala
405 410 415

Leu Tyr Pro Leu Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly
420 425 430

Ile Pro Gln Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn
435 440 445

His Ser Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe
450 455 460

Ile Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr
465 470 475 480

Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr Leu
485 490 495

Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser
500 505 510

Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp
515 520 525

Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met Pro Thr Glu Gly
530 535 540

Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp
545 550 555 560

Val Thr Trp Ile Leu Leu Arg Thr Val Asn Asn Arg Thr Met His Tyr
565 570 575

Ser Ile Ser Lys Gln Lys Met Ala Ile Thr Lys Glu His Ser Ile Thr
580 585 590

Leu Asn Leu Thr Ile Met Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr
595 600 605

Ala Cys Arg Ala Arg Asn Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys
610 615 620

Lys Glu Ile Thr Ile Arg Gly Glu His Cys Asn Lys Lys Ala Val Phe
625 630 635 640

Ser Arg Ile Ser Lys Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln
645 650 655

Ser Asn Val Lys His
660

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 668 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ser Glu Gln Asn Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp
1 5 10 15

Leu Cys Val Glu Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser
20 25 30

Leu Asp Leu Pro Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys
35 40 45

Ala Asn Thr Thr Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp
50 55 60

Trp Leu Trp Pro Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val
65 70 75 80

Thr Glu Cys Ser Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys
85 90 95

Val Ile Gly Asn Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr
100 105 110

Asp Leu Ala Ser Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro
115 120 125

Phe Ile Ala Ser Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu
130 135 140

Asn Lys Asn Lys Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn
145 150 155 160

Leu Asn Val Ser Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro
165 170 175

Asp Gly Asn Arg Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro
180 185 190

Ser Tyr Met Ile Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile
195 200 205

Asn Asp Glu Ser Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly
210 215 220

Tyr Arg Ile Tyr Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu
225 230 235 240

Ser Val Gly Glu Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu
245 250 255

Asn Val Gly Ile Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln
260 265 270

His Lys Lys Leu Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu
275 280 285

Met Lys Lys Phe Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser
290 295 300

Asp Gln Gly Leu Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys
305 310 315 320

Lys Asn Ser Thr Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe
325 330 335

Gly Ser Gly Met Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val
340 345 350

Arg Ile Pro Ala Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp
355 360 365

Tyr Lys Asn Gly Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly
370 375 380

His Val Leu Thr Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr
385 390 395 400

Thr Val Ile Leu Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val
405 410 415

Val Ser Leu Val Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu
420 425 430

Ile Ser Pro Val Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr
435 440 445

Cys Thr Val Tyr Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp
450 455 460

Gln Leu Glu Glu Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val
465 470 475 480

Thr Asn Pro Tyr Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln
485 490 495

Gly Gly Asn Lys Ile Ala Val Asn Lys Asn Gln Phe Ala Leu Ile Glu
500 505 510

Gly Lys Asn Lys Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val
515 520 525

Ser Ala Leu Tyr Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu
530 535 540

Arg Val Ile Ser Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln
545 550 555 560

Pro Asp Met Gln Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr
565 570 575

Ala Asp Arg Ser Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro
580 585 590

Gln Pro Leu Pro Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys
595 600 605

Asn Leu Asp Thr Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser
610 615 620

Thr Asn Asp Ile Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp
625 630 635 640

Gln Gly Asp Tyr Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg
645 650 655

His Cys Val Val Arg Gln Leu Thr Val Leu Glu Arg
660 665

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 780 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu Cys Ala Leu Leu Ser
1 5 10 15

Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly Ser Lys Leu Lys Asp Pro
20 25 30

Glu Leu Ser Leu Lys Gly Thr Gln His Ile Met Gln Ala Gly Gln Thr
35 40 45

Leu His Leu Gln Cys Arg Gly Glu Ala Ala His Lys Trp Ser Leu Pro
50 55 60

Glu Met Val Ser Lys Glu Ser Glu Arg Leu Ser Ile Thr Lys Ser Ala
65 70 75 80

Cys Gly Arg Asn Gly Lys Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr
85 90 95

Ala Gln Ala Asn His Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val
100 105 110

Pro Thr Ser Lys Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile
115 120 125

Ser Asp Thr Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu
130 135 140

Ile Ile His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val
145 150 155 160

Thr Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr
165 170 175

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly Phe
180 185 190

Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr Cys Glu
195 200 205

Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu Thr His Arg
210 215 220

Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr Pro Arg Pro Val
225 230 235 240

Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn Cys Thr Ala Thr Thr
245 250 255

Pro Leu Asn Thr Arg Val Gln Met Thr Trp Ser Tyr Pro Asp Glu Lys
260 265 270

Asn Lys Arg Ala Ser Val Arg Arg Arg Ile Asp Gln Ser Asn Ser His
275 280 285

Ala Asn Ile Phe Tyr Ser Val Leu Thr Ile Asp Lys Met Gln Asn Lys
290 295 300

Asp Lys Gly Leu Tyr Thr Cys Arg Val Arg Ser Gly Pro Ser Phe Lys
305 310 315 320

Ser Val Asn Thr Ser Val His Ile Tyr Asp Lys Ala Phe Ile Thr Val
325 330 335

Lys His Arg Lys Gln Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser
340 345 350

Tyr Arg Leu Ser Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val
355 360 365

Trp Leu Lys Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu
370 375 380

Thr Arg Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala
385 390 395 400

Gly Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
405 410 415

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr Glu
420 425 430

Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu Gly Ser
435 440 445

Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln Pro Thr Ile
450 455 460

Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser Glu Ala Arg Cys
465 470 475 480

Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile Leu Asp Ala Asp Ser
485 490 495

Asn Met Gly Asn Arg Ile Glu Ser Ile Thr Gln Arg Met Ala Ile Ile
500 505 510

Glu Gly Lys Asn Lys Met Ala Ser Thr Leu Val Val Ala Asp Ser Arg
515 520 525

Ile Ser Gly Ile Tyr Ile Cys Ile Ala Ser Asn Lys Val Gly Thr Val
530 535 540

Gly Arg Asn Ile Ser Phe Tyr Ile Thr Asp Val Pro Asn Gly Phe His
545 550 555 560

Val Asn Leu Glu Lys Met Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser
565 570 575

Cys Thr Val Asn Lys Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu
580 585 590

Arg Thr Val Asn Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys
595 600 605

Met Ala Ile Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met
610 615 620

Asn Val Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn
625 630 635 640

Val Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg
645 650 655

Asp Gln Glu Ala Pro Tyr Leu Leu Arg Asn Leu Ser Asp His Thr Val
660 665 670

Ala Ile Ser Ser Ser Thr Thr Leu Asp Cys His Ala Asn Gly Val Pro
675 680 685

Glu Pro Gln Ile Thr Trp Phe Lys Asn Asn His Lys Ile Gln Gln Glu
690 695 700

Pro Gly Ile Ile Leu Gly Pro Gly Ser Ser Thr Leu Phe Ile Glu Arg
705 710 715 720

Val Thr Glu Glu Asp Glu Gly Val Tyr His Cys Lys Ala Thr Asn Gln
725 730 735

Lys Gly Ser Val Glu Ser Ser Ala Tyr Leu Thr Val Gln Gly Thr Ser
740 745 750

Asp Lys Ser Asn Leu Glu Leu Ile Thr Leu Thr Cys Thr Cys Val Ala
755 760 765

Ala Thr Leu Phe Trp Leu Leu Leu Thr Leu Leu Ile
770 775 780

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 788 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Gln Ser Lys Val Leu Leu Ala Val Ala Leu Trp Leu Cys Val Glu
1 5 10 15

Thr Arg Ala Ala Ser Val Gly Leu Pro Ser Val Ser Leu Asp Leu Pro
20 25 30

Arg Leu Ser Ile Gln Lys Asp Ile Leu Thr Ile Lys Ala Asn Thr Thr
35 40 45

Leu Gln Ile Thr Cys Arg Gly Gln Arg Asp Leu Asp Trp Leu Trp Pro
50 55 60

Asn Asn Gln Ser Gly Ser Glu Gln Arg Val Glu Val Thr Glu Cys Ser
65 70 75 80

Asp Gly Leu Phe Cys Lys Thr Leu Thr Ile Pro Lys Val Ile Gly Asn
85 90 95

Asp Thr Gly Ala Tyr Lys Cys Phe Tyr Arg Glu Thr Asp Leu Ala Ser
100 105 110

Val Ile Tyr Val Tyr Val Gln Asp Tyr Arg Ser Pro Phe Ile Ala Ser
115 120 125

Val Ser Asp Gln His Gly Val Val Tyr Ile Thr Glu Asn Lys Asn Lys
130 135 140

Thr Val Val Ile Pro Cys Leu Gly Ser Ile Ser Asn Leu Asn Val Ser
145 150 155 160

Leu Cys Ala Arg Tyr Pro Glu Lys Arg Phe Val Pro Asp Gly Asn Arg
165 170 175

Ile Ser Trp Asp Ser Lys Lys Gly Phe Thr Ile Pro Ser Tyr Met Ile
180 185 190

Ser Tyr Ala Gly Met Val Phe Cys Glu Ala Lys Ile Asn Asp Glu Ser
195 200 205

Tyr Gln Ser Ile Met Tyr Ile Val Val Val Val Gly Tyr Arg Ile Tyr
210 215 220

Asp Val Val Leu Ser Pro Ser His Gly Ile Glu Leu Ser Val Gly Glu
225 230 235 240

Lys Leu Val Leu Asn Cys Thr Ala Arg Thr Glu Leu Asn Val Gly Ile
245 250 255

Asp Phe Asn Trp Glu Tyr Pro Ser Ser Lys His Gln His Lys Lys Leu
260 265 270

Val Asn Arg Asp Leu Lys Thr Gln Ser Gly Ser Glu Met Lys Lys Phe
275 280 285

Leu Ser Thr Leu Thr Ile Asp Gly Val Thr Arg Ser Asp Gln Gly Leu
290 295 300

Tyr Thr Cys Ala Ala Ser Ser Gly Leu Met Thr Lys Lys Asn Ser Thr
305 310 315 320

Phe Val Arg Val His Glu Lys Pro Phe Val Ala Phe Gly Ser Gly Met
325 330 335

Glu Ser Leu Val Glu Ala Thr Val Gly Glu Arg Val Arg Ile Pro Ala
340 345 350

Lys Tyr Leu Gly Tyr Pro Pro Pro Glu Ile Lys Trp Tyr Lys Asn Gly
355 360 365

Ile Pro Leu Glu Ser Asn His Thr Ile Lys Ala Gly His Val Leu Thr
370 375 380

Ile Met Glu Val Ser Glu Arg Asp Thr Gly Asn Tyr Thr Val Ile Leu
385 390 395 400

Thr Asn Pro Ile Ser Lys Glu Lys Gln Ser His Val Val Ser Leu Val
405 410 415

Val Tyr Val Pro Pro Gln Ile Gly Glu Lys Ser Leu Ile Ser Pro Val
420 425 430

Asp Ser Tyr Gln Tyr Gly Thr Thr Gln Thr Leu Thr Cys Thr Val Tyr
435 440 445

Ala Ile Pro Pro Pro His His Ile His Trp Tyr Trp Gln Leu Glu Glu
450 455 460

Glu Cys Ala Asn Glu Pro Ser Gln Ala Val Ser Val Thr Asn Pro Tyr
465 470 475 480

Pro Cys Glu Glu Trp Arg Ser Val Glu Asp Phe Gln Gly Gly Asn Lys
485 490 495

Ile Ala Val Asn Lys Asn Gln Phe Ala Leu Ile Glu Gly Lys Asn Lys
500 505 510

Thr Val Ser Thr Leu Val Ile Gln Ala Ala Asn Val Ser Ala Leu Tyr
515 520 525

Lys Cys Glu Ala Val Asn Lys Val Gly Arg Gly Glu Arg Val Ile Ser
530 535 540

Phe His Val Thr Arg Gly Pro Glu Ile Thr Leu Gln Pro Asp Met Gln
545 550 555 560

Pro Thr Glu Gln Glu Ser Val Ser Leu Trp Cys Thr Ala Asp Arg Ser
565 570 575

Thr Phe Glu Asn Leu Thr Trp Tyr Lys Leu Gly Pro Gln Pro Leu Pro
580 585 590

Ile His Val Gly Glu Leu Pro Thr Pro Val Cys Lys Asn Leu Asp Thr
595 600 605

Leu Trp Lys Leu Asn Ala Thr Met Phe Ser Asn Ser Thr Asn Asp Ile
610 615 620

Leu Ile Met Glu Leu Lys Asn Ala Ser Leu Gln Asp Gln Gly Asp Tyr
625 630 635 640

Val Cys Leu Ala Gln Asp Arg Lys Thr Lys Lys Arg His Cys Val Val
645 650 655

Arg Gln Leu Thr Val Leu Glu Arg Val Ala Pro Thr Ile Thr Gly Asn
660 665 670

Leu Glu Asn Gln Thr Thr Ser Ile Gly Glu Ser Ile Glu Val Ser Cys
675 680 685

Thr Ala Ser Gly Asn Pro Pro Pro Gln Ile Met Trp Phe Lys Asp Asn
690 695 700

Glu Thr Leu Val Glu Asp Ser Gly Ile Val Leu Lys Asp Gly Asn Arg
705 710 715 720

Asn Leu Thr Ile Arg Arg Val Arg Lys Glu Asp Glu Gly Leu Tyr Cys
725 730 735

Gln Ala Cys Ser Val Leu Gly Cys Ala Lys Val Glu Ala Phe Phe Ile
740 745 750

Ile Glu Gly Ala Gln Glu Lys Thr Asn Leu Glu Ile Ile Ile Leu Val
755 760 765

Gly Thr Thr Val Ile Ala Met Phe Phe Trp Leu Leu Leu Val Ile Ile
770 775 780

Leu Gly Thr Val
785

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2264 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
GGTGTGGTCG CTGCGTTTCC TCTGCCTGCG CCGGGCATCA CTTGCGCGCC GCAGAAAGTC    60
CGTCTGGCAG CCTGGATATC CTCTCCTACC GGCACCCGCA GACGCCCCTG CAGCCGCGGT   120
CGGCGCCCGG GCTCCCTAGC CCTGTGCGCT CAACTGTCCT GCGCTGCGGG GTGCCGCGAG   180
TTCCACCTCC GCGCCTCCTT CTCTAGACAG GCGCTGGGAG AAAGAACCGG CTCCCGAGTT   240
CCGGCATTTC GCCCGGCTCG AGGTGCAGGA TGCAGAGCAA GGTGCTGCTG GCCGTCGCCC   300
TGTGGCTCTG CGTGGAGACC CGGGCCGCCT CTGTGGGTTT GCCTAGTGTT TCTCTTGATC   360
TGCCCAGGCT CAGCATACAA AAAGACATAC TTACAATTAA GGCTAATACA ACTCTTCAAA   420
TTACTTGCAG GGGACAGAGG GACTTGGACT GGCTTTGGCC CAATAATCAG AGTGGCAGTG   480
AGCAAAGGGT GGAGGTGACT GAGTGCAGCG ATGGCCTCTT CTGTAAGACA CTCACAATTC   540
CAAAAGTGAT CGGAAATGAC ACTGGAGCCT ACAAGTGCTT CTACCGGGAA ACTGACTTGG   600
CCTCGGTCAT TTATGTCTAT GTTCAAGATT ACAGATCTCC ATTTATTGCT TCTGTTAGTG   660
ACCAACATGG AGTCGTGTAC ATTACTGAGA ACAAAAACAA AACTGTGGTG ATTCCATGTC   720
TCGGGTCCAT TTCAAATCTC AACGTGTCAC TTTGTGCAAG ATACCCAGAA AAGAGATTTG   780
TTCCTGATGG TAACAGAATT TCCTGGGACA GCAAGAAGGG CTTTACTATT CCCAGCTACA   840
TGATCAGCTA TGCTGGCATG GTCTTCTGTG AAGCAAAAAT TAATGATGAA AGTTACCAGT   900
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CTATTATGTA CATAGTTGTC GTTGTAGGGT ATAGGATTTA TGATGTGGTT CTGAGTCCGT 960

CTCATGGAAT TGAACTATCT GTTGGAGAAA AGCTTGTCTT AAATTGTACA GCAAGAACTG 1020

AACTAAATGT GGGGATTGAC TTCAACTGGG AATACCCTTC TTCGAAGCAT CAGCATAAGA 1080

AACTTGTAAA CCGAGACCTA AAAACCCAGT CTGGGAGTGA GATGAAGAAA TTTTTGAGCA 1140

CCTTAACTAT AGATGGTGTA ACCCGGAGTG ACCAAGGATT GTACACCTGT GCAGCATCCA 1200

GTGGGCTGAT GACCAAGAAG AACAGCACAT TTGTCAGGGT CCATGAAAAA CCTTTTGTTG 1260

CTTTTGGAAG TGGCATGGAA TCTCTGGTGG AAGCCACGGT GGGGGAGCGT GTCAGAATCC 1320

CTGCGAAGTA CCTTGGTTAC CCACCCCCAG AAATAAAATG GTATAAAAAT GGAATACCCC 1380

TTGAGTCCAA TCACACAATT AAAGCGGGGC ATGTACTGAC GATTATGGAA GTGAGTGAAA 1440

GAGACACAGG AAATTACACT GTCATCCTTA CCAATCCCAT TTCAAAGGAG AAGCAGAGCC 1500

ATGTGGTCTC TCTGGTTGTG TATGTCCCAC CCCAGATTGG TGAGAAATCT CTAATCTCTC 1560

CTGTGGATTC CTACCAGTAC GGCACCACTC AAACGCTGAC ATGTACGGTC TATGCCATTC 1620

CTCCCCCGCA TCACATCCAC TGGTATTGGC AGTTGGAGGA AGAGTGCGCC AACGAGCCCA 1680

GCCAAGCTGT CTCAGTGACA AACCCATACC CTTGTGAAGA ATGGAGAAGT GTGGAGGACT 1740

TCCAGGGAGG AAATAAAATT GCCGTTAATA AAAATCAATT TGCTCTAATT GAAGGAAAAA 1800

ACAAAACTGT AAGTACCCTT GTTATCCAAG CGGCAAATGT GTCAGCTTTG TACAAATGTG 1860

AAGCGGTCAA CAAAGTCGGG AGAGGAGAGA GGGTGATCTC CTTCCACGTG ACCAGGGGTC 1920

CTGAAATTAC TTTGCAACCT GACATGCAGC CCACTGAGCA GGAGAGCGTG TCTTTGTGGT 1980

GCACTGCAGA CAGATCTACG TTTGAGAACC TCACATGGTA CAAGCTTGGC CCACAGCCTC 2040

TGCCAATCCA TGTGGGAGAG TTGCCCACAC CTGTTTGCAA GAACTTGGAT ACTCTTTGGA 2100

AATTGAATGC CACCATGTTC TCTAATAGCA CAAATGACAT TTTGATCATG GAGCTTAAGA 2160

ATGCATCCTT GCAGGACCAA GGAGACTATG TCTGCCTTGC TCAAGACAGG AAGACCAAGA 2220

AAAGACATTG CGTGGTCAGG CAGCTCACAG TCCTAGAGCG TTAA 2264

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2352 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GCGCTCACCA TGGTCAGCTA CTGGGACACC GGGGTCCTGC TGTGCGCGCT GCTCAGCTGT 60

CTGCTTCTCA CAGGATCTAG TTCAGGTTCA AAATTAAAAG ATCCTGAACT GAGTTTAAAA 120

GGCACCCAGC ACATCATGCA AGCAGGCCAG ACACTGCATC TCCAATGCAG GGGGGAAGCA 180

GCCCATAAAT GGTCTTTGCC TGAAATGGTG AGTAAGGAAA GCGAAAGGCT GAGCATAACT 240

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AAATCTGCCT GTGGAAGAAA TGGCAAACAA TTCTGCAGTA CTTTAACCTT GAACACAGCT   300
CAAGCAAACC ACACTGGCTT CTACAGCTGC AAATATCTAG CTGTACCTAC TTCAAAGAAG   360
AAGGAAACAG AATCTGCAAT CTATATATTT ATTAGTGATA CAGGTAGACC TTTCGTAGAG   420
ATGTACAGTG AAATCCCCGA AATTATACAC ATGACTGAAG GAAGGGAGCT CGTCATTCCC   480
TGCCGGGTTA CGTCACCTAA CATCACTGTT ACTTTAAAAA AGTTTCCACT TGACACTTTG   540
ATCCCTGATG GAAAACGCAT AATCTGGGAC AGTAGAAAGG GCTTCATCAT ATCAAATGCA   600
ACGTACAAAG AAATAGGGCT TCTGACCTGT GAAGCAACAG TCAATGGGCA TTTGTATAAG   660
ACAAACTATC TCACACATCG ACAAACCAAT ACAATCATAG ATGTCCAAAT AAGCACACCA   720
CGCCCAGTCA AATTACTTAG AGGCCATACT CTTGTCCTCA ATTGTACTGC TACCACTCCC   780
TTGAACACGA GAGTTCAAAT GACCTGGAGT TACCCTGATG AAAAAAATAA GAGAGCTTCC   840
GTAAGGCGAC GAATTGACCA AAGCAATTCC CATGCCAACA TATTCTACAG TGTTCTTACT   900
ATTGACAAAA TGCAGAACAA AGACAAAGGA CTTTATACTT GTCGTGTAAG GAGTGGACCA   960
TCATTCAAAT CTGTTAACAC CTCAGTGCAT ATATATGATA AAGCATTCAT CACTGTGAAA  1020
CATCGAAAAC AGCAGGTGCT TGAAACCGTA GCTGGCAAGC GGTCTTACCG GCTCTCTATG  1080
AAAGTGAAGG CATTTCCCTC GCCGGAAGTT GTATGGTTAA AAGATGGGTT ACCTGCGACT  1140
GAGAAATCTG CTCGCTATTT GACTCGTGGC TACTCGTTAA TTATCAAGGA CGTAACTGAA  1200
GAGGATGCAG GGAATTATAC AATCTTGCTG AGCATAAAAC AGTCAAATGT GTTTAAAAAC  1260
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CTCACTGCCA CTCTAATTGT CAATGTGAAA CCCCAGATTT ACGAAAAGGC CGTGTCATCG 1320

TTTCCAGACC CGGCTCTCTA CCCACTGGGC AGCAGACAAA TCCTGACTTG TACCGCATAT 1380

GGTATCCCTC AACCTACAAT CAAGTGGTTC TGGCACCCCT GTAACCATAA TCATTCCGAA 1440

GCAAGGTGTG ACTTTTGTTC CAATAATGAA GAGTCCTTTA TCCTGGATGC TGACAGCAAC 1500

ATGGGAAACA GAATTGAGAG CATCACTCAG CGCATGGCAA TAATAGAAGG AAAGAATAAG 1560

ATGGCTAGCA CCTTGGTTGT GGCTGACTCT AGAATTTCTG GAATCTACAT TTGCATAGCT 1620

TCCAATAAAG TTGGGACTGT GGGAAGAAAC ATAAGCTTTT ATATCACAGA TGTGCCAAAT 1680

GGGTTTCATG TTAACTTGGA AAAAATGCCG ACGGAAGGAG AGGACCTGAA ACTGTCTTGC 1740

ACAGTTAACA AGTTCTTATA CAGAGACGTT ACTTGGATTT TACTGCGGAC AGTTAATAAC 1800

AGAACAATGC ACTACAGTAT TAGCAAGCAA AAAATGGCCA TCACTAAGGA GCACTCCATC 1860

ACTCTTAATC TTACCATCAT GAATGTTTCC CTGCAAGATT CAGGCACCTA TGCCTGCAGA 1920

GCCAGGAATG TATACACAGG GGAAGAAATC CTCCAGAAGA AAGAAATTAC AATCAGAGAT 1980

CAGGAAGCAC CATACCTCCT GCGAAACCTC AGTGATCACA CAGTGGCCAT CAGCAGTTCC 2040

ACCACTTTAG ACTGTCATGC TAATGGTGTC CCCGAGCCTC AGATCACTTG GTTTAAAAAC 2100

AACCACAAAA TACAACAAGA GCCTGGAATT ATTTTAGGAC CAGGAAGCAG CACGCTGTTT 2160

ATTGAAAGAG TCACAGAAGA GGATGAAGGT GTCTATCACT GCAAAGCCAC CAACCAGAAG 2220

GGCTCTGTGG AAAGTTCAGC ATACCTCACT GTTCAAGGAA CCTCGGACAA GTCTAATCTG 2280

GAGCTGATCA CTCTAACATG CACCTGTGTG GCTGCGACTC TCTTCTGGCT CCTATTAACC 2340

CTCCTTATCT AA 2352

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2383 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCGAGGTGC AGGATGCAGA GCAAGGTGCT GCTGGCCGTC GCCCTGTGGC TCTGCGTGGA 60

GACCCGGGCC GCCTCTGTGG GTTTGCCTAG TGTTTCTCTT GATCTGCCCA GGCTCAGCAT 120

ACAAAAAGAC ATACTTACAA TTAAGGCTAA TACAACTCTT CAAATTACTT GCAGGGGACA 180

GAGGGACTTG GACTGGCTTT GGCCCAATAA TCAGAGTGGC AGTGAGCAAA GGGTGGAGGT 240

GACTGAGTGC AGCGATGGCC TCTTCTGTAA GACACTCACA ATTCCAAAAG TGATCGGAAA 300

TGACACTGGA GCCTACAAGT GCTTCTACCG GGAAACTGAC TTGGCCTCGG TCATTTATGT 360

CTATGTTCAA GATTACAGAT CTCCATTTAT TGCTTCTGTT AGTGACCAAC ATGGAGTCGT 420

GTACATTACT GAGAACAAAA ACAAAACTGT GGTGATTCCA TGTCTCGGGT CCATTTCAAA 480

TCTCAACGTG TCACTTTGTG CAAGATACCC AGAAAAGAGA TTTGTTCCTG ATGGTAACAG 540

AATTTCCTGG GACAGCAAGA AGGGCTTTAC TATTCCCAGC TACATGATCA GCTATGCTGG 600

CATGGTCTTC TGTGAAGCAA AAATTAATGA TGAAAGTTAC CAGTCTATTA TGTACATAGT 660

TGTCGTTGTA GGGTATAGGA TTTATGATGT GGTTCTGAGT CCGTCTCATG GAATTGAACT 720

ATCTGTTGGA GAAAAGCTTG TCTTAAATTG TACAGCAAGA ACTGAACTAA ATGTGGGGAT 780

TGACTTCAAC TGGGAATACC CTTCTTCGAA GCATCAGCAT AAGAAACTTG TAAACCGAGA 840

CCTAAAAACC CAGTCTGGGA GTGAGATGAA GAAATTTTTG AGCACCTTAA CTATAGATGG 900

TGTAACCCGG AGTGACCAAG GATTGTACAC CTGTGCAGCA TCCAGTGGGC TGATGACCAA 960

GAAGAACAGC ACATTTGTCA GGGTCCATGA AAAACCTTTT GTTGCTTTTG GAAGTGGCAT 1020

GGAATCTCTG GTGGAAGCCA CGGTGGGGGA GCGTGTCAGA ATCCCTGCGA AGTACCTTGG 1080

TTACCCACCC CCAGAAATAA AATGGTATAA AAATGGAATA CCCCTTGAGT CCAATCACAC 1140

AATTAAAGCG GGGCATGTAC TGACGATTAT GGAAGTGAGT GAAAGAGACA CAGGAAATTA 1200

CACTGTCATC CTTACCAATC CCATTTCAAA GGAGAAGCAG AGCCATGTGG TCTCTCTGGT 1260

TGTGTATGTC CCACCCCAGA TTGGTGAGAA ATCTCTAATC TCTCCTGTGG ATTCCTACCA 1320

GTACGGCACC ACTCAAACGC TGACATGTAC GGTCTATGCC ATTCCTCCCC CGCATCACAT 1380

CCACTGGTAT TGGCAGTTGG AGGAAGAGTG CGCCAACGAG CCCAGCCAAG CTGTCTCAGT 1440

GACAAACCCA TACCCTTGTG AAGAATGGAG AAGTGTGGAG GACTTCCAGG GAGGAAATAA 1500

AATTGCCGTT AATAAAAATC AATTTGCTCT AATTGAAGGA AAAAACAAAA CTGTAAGTAC 1560

CCTTGTTATC CAAGCGGCAA ATGTGTCAGC TTTGTACAAA TGTGAAGCGG TCAACAAAGT 1620

CGGGAGAGGA GAGAGGGTGA TCTCCTTCCA CGTGACCAGG GGTCCTGAAA TTACTTTGCA 1680

ACCTGACATG CAGCCCACTG AGCAGGAGAG CGTGTCTTTG TGGTGCACTG CAGACAGATC 1740

TACGTTTGAG AACCTCACAT GGTACAAGCT TGGCCCACAG CCTCTGCCAA TCCATGTGGG 1800

AGAGTTGCCC ACACCTGTTT GCAAGAACTT GGATACTCTT TGGAAATTGA ATGCCACCAT 1860

GTTCTCTAAT AGCACAAATG ACATTTTGAT CATGGAGCTT AAGAATGCAT CCTTGCAGGA 1920

CCAAGGAGAC TATGTCTGCC TTGCTCAAGA CAGGAAGACC AAGAAAAGAC ATTGCGTGGT 1980

CAGGCAGCTC ACAGTCCTAG AGCGTGTGGC ACCCACGATC ACAGGAAACC TGGAGAATCA 2040

GACGACAAGT ATTGGGGAAA GCATCGAAGT CTCATGCACG GCATCTGGGA ATCCCCCTCC 2100

ACAGATCATG TGGTTTAAAG ATAATGAGAC CCTTGTAGAA GACTCAGGCA TTGTATTGAA 2160

GGATGGGAAC CGGAACCTCA CTATCCGCAG AGTGAGGAAG GAGGACGAAG GCCTCTACAC 2220

CTGCCAGGCA TGCAGTGTTC TTGGCTGTGC AAAAGTGGAG GCATTTTTCA TAATAGAAGG 2280

TGCCCAGGAA AAGACGAACT TGGAAATCAT TATTCTAGTA GGCACGACGG TGATTGCCAT 2340

GTTCTTCTGG CTACTTCTTG TCATCATCCT AGGGACCGTT TAA 2383

WHAT IS CLAIMED IS:

1. A soluble VEGF inhibitor in substantially pure form which specifically binds VEGF and inhibits cellular VEGF receptor activity.

2. The soluble VEGF inhibitor according to Claim 1 wherein the soluble VEGF receptor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI and sVEGF-RTMII.

3. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RI comprising the amino acid sequence:

Met Val Ser Tyr Trp Asp Thr Gly Val Leu Leu

Cys Ala Leu Leu Ser Cys Leu Leu Leu Thr Gly Ser Ser Ser Gly

Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His

Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu

Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser

Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys

Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His

Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys

Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr

Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile

His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr

Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly

Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr

Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu

Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr

Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn

Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp

Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg

Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu

Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys

Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val

His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln

Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser

Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys

Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg

Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly

Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys

Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr

Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu

Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln

Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser

Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile

Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr

Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr

Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile

Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr

Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met

Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys

Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn

Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile

Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val

Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val

Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg

Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys

Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys

His. (SEQ. ID. NO.: 6)

4. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RI comprising the amino acid sequence:

Ser Lys Leu Lys Asp Pro Glu Leu Ser Leu Lys Gly Thr Gln His
Ile Met Gln Ala Gly Gln Thr Leu His Leu Gln Cys Arg Gly Glu
Ala Ala His Lys Trp Ser Leu Pro Glu Met Val Ser Lys Glu Ser
Glu Arg Leu Ser Ile Thr Lys Ser Ala Cys Gly Arg Asn Gly Lys
Gln Phe Cys Ser Thr Leu Thr Leu Asn Thr Ala Gln Ala Asn His
Thr Gly Phe Tyr Ser Cys Lys Tyr Leu Ala Val Pro Thr Ser Lys
Lys Lys Glu Thr Glu Ser Ala Ile Tyr Ile Phe Ile Ser Asp Thr
Gly Arg Pro Phe Val Glu Met Tyr Ser Glu Ile Pro Glu Ile Ile
His Met Thr Glu Gly Arg Glu Leu Val Ile Pro Cys Arg Val Thr

Ser Pro Asn Ile Thr Val Thr Leu Lys Lys Phe Pro Leu Asp Thr

Leu Ile Pro Asp Gly Lys Arg Ile Ile Trp Asp Ser Arg Lys Gly

Phe Ile Ile Ser Asn Ala Thr Tyr Lys Glu Ile Gly Leu Leu Thr

Cys Glu Ala Thr Val Asn Gly His Leu Tyr Lys Thr Asn Tyr Leu

Thr His Arg Gln Thr Asn Thr Ile Ile Asp Val Gln Ile Ser Thr

Pro Arg Pro Val Lys Leu Leu Arg Gly His Thr Leu Val Leu Asn

Cys Thr Ala Thr Thr Pro Leu Asn Thr Arg Val Gln Met Thr Trp

Ser Tyr Pro Asp Glu Lys Asn Lys Arg Ala Ser Val Arg Arg Arg

Ile Asp Gln Ser Asn Ser His Ala Asn Ile Phe Tyr Ser Val Leu

Thr Ile Asp Lys Met Gln Asn Lys Asp Lys Gly Leu Tyr Thr Cys

Arg Val Arg Ser Gly Pro Ser Phe Lys Ser Val Asn Thr Ser Val
His Ile Tyr Asp Lys Ala Phe Ile Thr Val Lys His Arg Lys Gln
Gln Val Leu Glu Thr Val Ala Gly Lys Arg Ser Tyr Arg Leu Ser
Met Lys Val Lys Ala Phe Pro Ser Pro Glu Val Val Trp Leu Lys
Asp Gly Leu Pro Ala Thr Glu Lys Ser Ala Arg Tyr Leu Thr Arg
Gly Tyr Ser Leu Ile Ile Lys Asp Val Thr Glu Glu Asp Ala Gly
Asn Tyr Thr Ile Leu Leu Ser Ile Lys Gln Ser Asn Val Phe Lys
Asn Leu Thr Ala Thr Leu Ile Val Asn Val Lys Pro Gln Ile Tyr
Glu Lys Ala Val Ser Ser Phe Pro Asp Pro Ala Leu Tyr Pro Leu
Gly Ser Arg Gln Ile Leu Thr Cys Thr Ala Tyr Gly Ile Pro Gln

Pro Thr Ile Lys Trp Phe Trp His Pro Cys Asn His Asn His Ser

Glu Ala Arg Cys Asp Phe Cys Ser Asn Asn Glu Glu Ser Phe Ile

Leu Asp Ala Asp Ser Asn Met Gly Asn Arg Ile Glu Ser Ile Thr

Gln Arg Met Ala Ile Ile Glu Gly Lys Asn Lys Met Ala Ser Thr

Leu Val Val Ala Asp Ser Arg Ile Ser Gly Ile Tyr Ile Cys Ile

Ala Ser Asn Lys Val Gly Thr Val Gly Arg Asn Ile Ser Phe Tyr

Ile Thr Asp Val Pro Asn Gly Phe His Val Asn Leu Glu Lys Met

Pro Thr Glu Gly Glu Asp Leu Lys Leu Ser Cys Thr Val Asn Lys

Phe Leu Tyr Arg Asp Val Thr Trp Ile Leu Leu Arg Thr Val Asn

Asn Arg Thr Met His Tyr Ser Ile Ser Lys Gln Lys Met Ala Ile

Thr Lys Glu His Ser Ile Thr Leu Asn Leu Thr Ile Met Asn Val

Ser Leu Gln Asp Ser Gly Thr Tyr Ala Cys Arg Ala Arg Asn Val

Tyr Thr Gly Glu Glu Ile Leu Gln Lys Lys Glu Ile Thr Ile Arg

Gly Glu His Cys Asn Lys Lys Ala Val Phe Ser Arg Ile Ser Lys

Phe Lys Ser Thr Arg Asn Asp Cys Thr Thr Gln Ser Asn Val Lys

His. (SEQ. ID. NO.: 12)

5. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RII comprising the amino acid sequence:

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQR
DLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVI
YVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFV
PDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVL
SPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEA
TVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVI
LTNPISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHI
HWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIAVNKNQFALIEGKNKT
VSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVSLW
CTADRSTFENLTWYKLGPQPLPIHVGELPTPVCKNLDTLWKLNATMFSNSTNDILIM
ELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLER. (SEQ.ID.NO.: 13)

6. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RTMI comprising the amino acid sequence:

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQCRGEA
AHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYSCKYLAVPT
SKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSPNITVTLKK
FPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYLTHRQTNT
IIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKRASVRRRIDQS
NSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIYDKAFITVKHRKQQ
VLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSARYLTRGYSLIIKDVTEED
AGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFPDPALYPLGSRQILTCTA
YGIPQPTIKWFWHPCNHNHSEARCDFCSNNEESFILDADSNMGNRIESITQRMAIIE
GKNKMASTLVVADSRISGIYICIASNKVGTVGRNISFYITDVPNGFHVNLEKMPTEG
EDLKLSCTVNKFLYRDVTWILLRTVNNRTMHYSISKQKMAITKEHSITLNLTIMNVS
LQDSGTYACRARNVYTGEEILQKKEITIRDQEAPYLLRNLSDHTVAISSSTTLDCHA
NGVPEPQITWFKNNHKIQQEPGIILGPGSSTLFIERVTEEDEGVYHCKATNQKGSVE
SSAYLTVQGTSDKSNLELITLTCTCVAATLFWLLLTLLI. (SEQ. ID. NO.: 14)

7. The soluble VEGF inhibitor of Claim 2 corresponding to sVEGF-RTMII comprising the amino acid sequence:

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQR
DLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVI
YVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFV
PDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVL
SPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEA
TVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVI
LTNPISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYGTTQTLTCTVYAIPPPHHI

HWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKIAVNKNQFALIEGKNKT
VSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVSLW
CTADRSTFENLTWYKLGPQPLPIHVGELPTPVCKNLDTLWKLNATMFSNSTNDILIM
ELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTITGNLENQTTSIGESI
EVSCTASGNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIRRVRKEDEGLYCQACSV
LGCAKVEAFFIIEGAQEKTNLEIIILVGTTVIAMFFWLLLVIILGTV. (SEQ. ID. NO.: 15)

8. An expression vector comprising a promoter, and a DNA sequence encoding a soluble VEGF inhibitor for expression in recombinant host cells wherein the soluble VEGF inhibitor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI and sVEGF-RTMII.

9. The expression vector of Claim 8 wherein the DNA encoding the sVEGF-RI comprises the nucleotide sequence:

GCGGACACTCCTCTCGGCTCCTCCCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGG

CTCGGGTGCAGCGGCCAGCGGGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTC

CTGGCTGGAGCCGCGAGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCGAACGAGAGG

ACGGACTCTGGCGGCCGGGTCGTTGGCCGGGGGAGCGCGGGCACCGGGCGAGCAGGCCG

CGTCGCGCTCACC ATG GTC AGC TAC TGG GAC ACC GGG GTC CTG CTG
TGC GCG CTG CTC AGC TGT CTG CTT CTC ACA GGA TCT AGT TCA GGT
TCA AAA TTA AAA GAT CCT GAA CTG AGT TTA AAA GGC ACC CAG CAC
ATC ATG CAA GCA GGC CAG ACA CTG CAT CTC CAA TGC AGG GGG GAA
GCA GCC CAT AAA TGG TCT TTG CCT GAA ATG GTG AGT AAG GAA AGC
GAA AGG CTG AGC ATA ACT AAA TCT GCC TGT GGA AGA AAT GGC AAA
CAA TTC TGC AGT ACT TTA ACC TTG AAC ACA GCT CAA GCA AAC CAC
ACT GGC TTC TAC AGC TGC AAA TAT CTA GCT GTA CCT ACT TCA AAG
AAG AAG GAA ACA GAA TCT GCA ATC TAT ATA TTT ATT AGT GAT ACA
GGT AGA CCT TTC GTA GAG ATG TAC AGT GAA ATC CCC GAA ATT ATA

CAC ATG ACT GAA GGA AGG GAG CTC GTC ATT CCC TGC CGG GTT ACG TCA CCT AAC ATC ACT GTT ACT TTA AAA AAG TTT CCA CTT GAC ACT TTG ATC CCT GAT GGA AAA CGC ATA ATC TGG GAC AGT AGA AAG GGC TTC ATC ATA TCA AAT GCA ACG TAC AAA GAA ATA GGG CTT CTG ACC TGT GAA GCA ACA GTC AAT GGG CAT TTG TAT AAG ACA AAC TAT CTC ACA CAT CGA CAA ACC AAT ACA ATC ATA GAT GTC CAA ATA AGC ACA CCA CGC CCA GTC AAA TTA CTT AGA GGC CAT ACT CTT GTC CTC AAT TGT ACT GCT ACC ACT CCC TTG AAC ACG AGA GTT CAA ATG ACC TGG AGT TAC CCT GAT GAA AAA AAT AAG AGA GCT TCC GTA AGG CGA CGA ATT GAC CAA AGC AAT TCC CAT GCC AAC ATA TTC TAC AGT GTT CTT

ACT ATT GAC AAA ATG CAG AAC AAA GAC AAA GGA CTT TAT ACT TGT
CGT GTA AGG AGT GGA CCA TCA TTC AAA TCT GTT AAC ACC TCA GTG
CAT ATA TAT GAT AAA GCA TTC ATC ACT GTG AAA CAT CGA AAA CAG
CAG GTG CTT GAA ACC GTA GCT GGC AAG CGG TCT TAC CGG CTC TCT
ATG AAA GTG AAG GCA TTT CCC TCG CCG GAA GTT GTA TGG TTA AAA
GAT GGG TTA CCT GCG ACT GAG AAA TCT GCT CGC TAT TTG ACT CGT
GGC TAC TCG TTA ATT ATC AAG GAC GTA ACT GAA GAG GAT GCA GGG
AAT TAT ACA ATC TTG CTG AGC ATA AAA CAG TCA AAT GTG TTT AAA
AAC CTC ACT GCC ACT CTA ATT GTC AAT GTG AAA CCC CAG ATT TAC
GAA AAG GCC GTG TCA TCG TTT CCA GAC CCG GCT CTC TAC CCA CTG

GGC AGC AGA CAA ATC CTG ACT TGT ACC GCA TAT GGT ATC CCT CAA

CCT ACA ATC AAG TGG TTC TGG CAC CCC TGT AAC CAT AAT CAT TCC

GAA GCA AGG TGT GAC TTT TGT TCC AAT AAT GAA GAG TCC TTT ATC

CTG GAT GCT GAC AGC AAC ATG GGA AAC AGA ATT GAG AGC ATC ACT

CAG CGC ATG GCA ATA ATA GAA GGA AAG AAT AAG ATG GCT AGC ACC

TTG GTT GTG GCT GAC TCT AGA ATT TCT GGA ATC TAC ATT TGC ATA

GCT TCC AAT AAA GTT GGG ACT GTG GGA AGA AAC ATA AGC TTT TAT

ATC ACA GAT GTG CCA AAT GGG TTT CAT GTT AAC TTG GAA AAA ATG

CCG ACG GAA GGA GAG GAC CTG AAA CTG TCT TGC ACA GTT AAC AAG

TTC TTA TAC AGA GAC GTT ACT TGG ATT TTA CTG CGG ACA GTT AAT

AAC AGA ACA ATG CAC TAC AGT ATT AGC AAG CAA AAA ATG GCC ATC

ACT AAG GAG CAC TCC ATC ACT CTT AAT CTT ACC ATC ATG AAT GTT

TCC CTG CAA GAT TCA GGC ACC TAT GCC TGC AGA GCC AGG AAT GTA

TAC ACA GGG GAA GAA ATC CTC CAG AAG AAA GAA ATT ACA ATC AGA

GGT GAG CAC TGC AAC AAA AAG GCT GTT TTC TCT CGG ATC TCC AAA

TTT AAA AGC ACA AGG AAT GAT TGT ACC ACA CAA AGT AAT GTA AAA

CAT TAA AGGACTCATTAAAAAGTAACAGTTGTCTCATATCATCTTGATTTATTGTCA

CTGTTGCTAACTTTCAGGCTCGGAGGAGATGCTCCTCCCAAAATGAGTTCGGAGATGAT

AGCAGTAATAATGAGACCCCCGGGCTCCAGCTCTGGGCCCCCCATTCAGGCCGAGGGGG

CTGCTCCGGGGGGCCGACTTGGTGCACGTTTGGATTTGGAGGATCCCTGCACTGCCTTC
TCTGTGTTTGTTGCTCTTGCTGTTTTCTCCTGCCTGATAAACAACAACTTGGGATGATC
CTTTCCATTTTGATGCCAACCTCTTTTTATTTTTAAGCGGCGCCCTATAGT.
(SEQ. ID. NO.: 5)

10. The expression vector of Claim 8 wherein the DNA encoding the sVEGF-RII comprises the nucleotide sequence:

GGTGTGGTCGCTGCGTTTCCTCTGCCTGCGCCGGGCATCACTTGCGCGCCGCAGAA
AGTCCGTCTGGCAGCCTGGATATCCTCTCCTACCGGCACCCGCAGACGCCCCTGCA
GCCGCGGTCGGCGCCCGGGCTCCCTAGCCCTGTGCGCTCAACTGTCCTGCGCTGCG
GGGTGCCGCGAGTTCCACCTCCGCGCCTCCTTCTCTAGACAGGCGCTGGGAGAAAG
AACCGGCTCCCGAGTTCCGGCATTTCGCCCGGCTCGAGGTGCAGGATGCAGAGCAA
GGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCGTGGAGACCCGGGCCGCCTCTGTGG
GTTTGCCTAGTGTTTCTCTTGATCTGCCCAGGCTCAGCATACAAAAAGACATACTT
ACAATTAAGGCTAATACAACTCTTCAAATTACTTGCAGGGGACAGAGGGACTTGGA
CTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAGCAAAGGGTGGAGGTGACTGAGT
GCAGCGATGGCCTCTTCTGTAAGACACTCACAATTCCAAAAGTGATCGGAAATGAC
ACTGGAGCCTACAAGTGCTTCTACCGGGAAACTGACTTGGCCTCGGTCATTTATGT
CTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAG
TCGTGTACATTACTGAGAACAAAAACAAAACTGTGGTGATTCCATGTCTCGGGTCC
ATTTCAAATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAAGAGATTTGTTCC
TGATGGTAACAGAATTTCCTGGGACAGCAAGAAGGGCTTTACTATTCCCAGCTACA
TGATCAGCTATGCTGGCATGGTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTAC
CAGTCTATTATGTACATAGTTGTCGTTGTAGGGTATAGGATTTATGATGTGGTTCT
GAGTCCGTCTCATGGAATTGAACTATCTGTTGGAGAAAAGCTTGTCTTAAATTGTA

CAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGGGAATACCCTTCTTCG
AAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCAGTCTGGGAGTGA
GATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGTGACCAAG
GATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTT
GTCAGGGTCCATGAAAAACCTTTTGTTGCTTTTGGAAGTGGCATGGAATCTCTGGT
GGAAGCCACGGTGGGGGAGCGTGTCAGAATCCCTGCGAAGTACCTTGGTTACCCAC
CCCCAGAAATAAAATGGTATAAAAATGGAATACCCCTTGAGTCCAATCACACAATT
AAAGCGGGGCATGTACTGACGATTATGGAAGTGAGTGAAAGAGACACAGGAAATTA
CACTGTCATCCTTACCAATCCCATTTCAAAGGAGAAGCAGAGCCATGTGGTCTCTC
TGGTTGTGTATGTCCCACCCCAGATTGGTGAGAAATCTCTAATCTCTCCTGTGGAT
TCCTACCAGTACGGCACCACTCAAACGCTGACATGTACGGTCTATGCCATTCCTCC
CCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGCGCCAACGAGCCCA
GCCAAGCTGTCTCAGTGACAAACCCATACCCTTGTGAAGAATGGAGAAGTGTGGAG
GACTTCCAGGGAGGAAATAAAATTGCCGTTAATAAAAATCAATTTGCTCTAATTGA
AGGAAAAAACAAAACTGTAAGTACCCTTGTTATCCAAGCGGCAAATGTGTCAGCTT
TGTACAAATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTGATCTCCTTC
CACGTGACCAGGGGTCCTGAAATTACTTTGCAACCTGACATGCAGCCCACTGAGCA
GGAGAGCGTGTCTTTGTGGTGCACTGCAGACAGATCTACGTTTGAGAACCTCACAT
GGTACAAGCTTGGCCCACAGCCTCTGCCAATCCATGTGGGAGAGTTGCCCACACCT
GTTTGCAAGAACTTGGATACTCTTTGGAAATTGAATGCCACCATGTTCTCTAATAG
CACAAATGACATTTTGATCATGGAGCTTAAGAATGCATCCTTGCAGGACCAAGGAG
ACTATGTCTGCCTTGCTCAAGACAGGAAGACCAAGAAAAGACATTGCGTGGTCAGG
CAGCTCACAGTCCTAGAGCGTTAA. (SEQ. ID. NO.: 16)

11. The expression vector of Claim 8 wherein the DNA encoding the sVEGF-RTMI comprises the nucleotide sequence:

GCGCTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAG
CTGTCTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGA

GTTTAAAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGC
AGGGGGGAAGCAGCCCATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGA
AAGGCTGAGCATAACTAAATCTGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTA
CTTTAACCTTGAACACAGCTCAAGCAAACCACACTGGCTTCTACAGCTGCAAATAT
CTAGCTGTACCTACTTCAAAGAAGAAGGAAACAGAATCTGCAATCTATATATTTAT
TAGTGATACAGGTAGACCTTTCGTAGAGATGTACAGTGAAATCCCCGAAATTATAC
ACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTCACCTAACATC
ACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACGCAT
AATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAG
GGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTC
ACACATCGACAAACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGT
CAAATTACTTAGAGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGA
ACACGAGAGTTCAAATGACCTGGAGTTACCCTGATGAAAAAAATAAGAGAGCTTCC
GTAAGGCGACGAATTGACCAAAGCAATTCCCATGCCAACATATTCTACAGTGTTCT
TACTATTGACAAAATGCAGAACAAAGACAAAGGACTTTATACTTGTCGTGTAAGGA
GTGGACCATCATTCAAATCTGTTAACACCTCAGTGCATATATATGATAAAGCATTC
ATCACTGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCAAGCGGTC
TTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTAA
AAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCG
TTAATTATCAAGGACGTAACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAG
CATAAAACAGTCAAATGTGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGA
AACCCCAGATTTACGAAAAGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCA
CTGGGCAGCAGACAAATCCTGACTTGTACCGCATATGGTATCCCTCAACCTACAAT
CAAGTGGTTCTGGCACCCCTGTAACCATAATCATTCCGAAGCAAGGTGTGACTTTT
GTTCCAATAATGAAGAGTCCTTTATCCTGGATGCTGACAGCAACATGGGAAACAGA
ATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAGAATAAGATGGCTAG
CACCTTGGTTGTGGCTGACTCTAGAATTTCTGGAATCTACATTTGCATAGCTTCCA
ATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCACAGATGTGCCAAAT
GGGTTTCATGTTAACTTGGAAAAAATGCCGACGGAAGGAGAGGACCTGAAACTGTC
TTGCACAGTTAACAAGTTCTTATACAGAGACGTTACTTGGATTTTACTGCGGACAG

TTAATAACAGAACAATGCACTACAGTATTAGCAAGCAAAAAATGGCCATCACTAAG
GAGCACTCCATCACTCTTAATCTTACCATCATGAATGTTTCCCTGCAAGATTCAGG
CACCTATGCCTGCAGAGCCAGGAATGTATACACAGGGGAAGAAATCCTCCAGAAGA
AAGAAATTACAATCAGAGATCAGGAAGCACCATACCTCCTGCGAAACCTCAGTGAT
CACACAGTGGCCATCAGCAGTTCCACCACTTTAGACTGTCATGCTAATGGTGTCCC
CGAGCCTCAGATCACTTGGTTTAAAAACAACCACAAAATACAACAAGAGCCTGGAA
TTATTTTAGGACCAGGAAGCAGCACGCTGTTTATTGAAAGAGTCACAGAAGAGGAT
GAAGGTGTCTATCACTGCAAAGCCACCAACCAGAAGGGCTCTGTGGAAAGTTCAGC
ATACCTCACTGTTCAAGGAACCTCGGACAAGTCTAATCTGGAGCTGATCACTCTAA
CATGCACCTGTGTGGCTGCGACTCTCTTCTGGCTCCTATTAACCCTCCTTATCTAA
. (SEQ. ID. NO.: 17)

12. The expression vector of Claim 8 wherein the DNA encoding the sVEGF-RTMII comprises the nucleotide sequence:

CTCGAGGTGCAGGATGCAGAGCAAGGTGCTGCTGGCCGTCGCCCTGTGGCTCTGCG
TGGAGACCCGGGCCGCCTCTGTGGGTTTGCCTAGTGTTTCTCTTGATCTGCCCAGG
CTCAGCATACAAAAAGACATACTTACAATTAAGGCTAATACAACTCTTCAAATTAC
TTGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTG
AGCAAAGGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCACA
ATTCCAAAAGTGATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAAAC
TGACTTGGCCTCGGTCATTTATGTCTATGTTCAAGATTACAGATCTCCATTTATTG
CTTCTGTTAGTGACCAACATGGAGTCGTGTACATTACTGAGAACAAAAACAAAACT
GTGGTGATTCCATGTCTCGGGTCCATTTCAAATCTCAACGTGTCACTTTGTGCAAG
ATACCCAGAAAAGAGATTTGTTCCTGATGGTAACAGAATTTCCTGGGACAGCAAGA
AGGGCTTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATGGTCTTCTGTGAA
GCAAAAATTAATGATGAAAGTTACCAGTCTATTATGTACATAGTTGTCGTTGTAGG
GTATAGGATTTATGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTG
GAGAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGAC

TTCAACTGGGAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGA
CCTAAAAACCCAGTCTGGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAG
ATGGTGTAACCCGGAGTGACCAAGGATTGTACACCTGTGCAGCATCCAGTGGGCTG
ATGACCAAGAAGAACAGCACATTTGTCAGGGTCCATGAAAAACCTTTTGTTGCTTT
TGGAAGTGGCATGGAATCTCTGGTGGAAGCCACGGTGGGGGAGCGTGTCAGAATCC
CTGCGAAGTACCTTGGTTACCCACCCCCAGAAATAAAATGGTATAAAAATGGAATA
CCCCTTGAGTCCAATCACACAATTAAAGCGGGGCATGTACTGACGATTATGGAAGT
GAGTGAAAGAGACACAGGAAATTACACTGTCATCCTTACCAATCCCATTTCAAAGG
AGAAGCAGAGCCATGTGGTCTCTCTGGTTGTGTATGTCCCACCCCAGATTGGTGAG
AAATCTCTAATCTCTCCTGTGGATTCCTACCAGTACGGCACCACTCAAACGCTGAC
ATGTACGGTCTATGCCATTCCTCCCCCGCATCACATCCACTGGTATTGGCAGTTGG
AGGAAGAGTGCGCCAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCCT
TGTGAAGAATGGAGAAGTGTGGAGGACTTCCAGGGAGGAAATAAAATTGCCGTTAA
TAAAAATCAATTTGCTCTAATTGAAGGAAAAAACAAAACTGTAAGTACCCTTGTTA
TCCAAGCGGCAAATGTGTCAGCTTTGTACAAATGTGAAGCGGTCAACAAAGTCGGG
AGAGGAGAGAGGGTGATCTCCTTCCACGTGACCAGGGGTCCTGAAATTACTTTGCA
ACCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTGGTGCACTGCAGACA
GATCTACGTTTGAGAACCTCACATGGTACAAGCTTGGCCCACAGCCTCTGCCAATC
CATGTGGGAGAGTTGCCCACACCTGTTTGCAAGAACTTGGATACTCTTTGGAAATT
GAATGCCACCATGTTCTCTAATAGCACAAATGACATTTTGATCATGGAGCTTAAGA
ATGCATCCTTGCAGGACCAAGGAGACTATGTCTGCCTTGCTCAAGACAGGAAGACC
AAGAAAAGACATTGCGTGGTCAGGCAGCTCACAGTCCTAGAGCGTGTGGCACCCAC
GATCACAGGAAACCTGGAGAATCAGACGACAAGTATTGGGGAAAGCATCGAAGTCT
CATGCACGGCATCTGGGAATCCCCCTCCACAGATCATGTGGTTTAAAGATAATGAG
ACCCTTGTAGAAGACTCAGGCATTGTATTGAAGGATGGGAACCGGAACCTCACTAT
CCGCAGAGTGAGGAAGGAGGACGAAGGCCTCTACACCTGCCAGGCATGCAGTGTTC
TTGGCTGTGCAAAAGTGGAGGCATTTTTCATAATAGAAGGTGCCCAGGAAAAGACG
AACTTGGAAATCATTATTCTAGTAGGCACGACGGTGATTGCCATGTTCTTCTGGCT
ACTTCTTGTCATCATCCTAGGGACCGTTTAA. (SEQ. ID. NO.: 18)

13. A recombinant host cell containing the expression vector of Claim 8.

14. A method for inhibiting VEGF receptor function comprising the administration of the VEGF inhibitor of Claim 1 in an amount sufficient to inhibit VEGF receptor function.

15. The method of Claim 14 wherein the VEGF inhibitor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI, and sVEGF-RTMII.

16. A pharmaceutical composition comprising the inhibitor of Claim 1 and a pharmaceutically acceptable carrier.

17. The pharmaceutical composition of Claim 16 wherein the inhibitor is selected from the group consisting of sVEGF-RI, sVEGF-RII, sVEGF-RTMI, and sVEGF-RTMII.

18. A method for inhibiting angiogenesis comprising the administration of the VEGF inhibitor of Claim 1 in an amount sufficient to inhibit angiogensis.

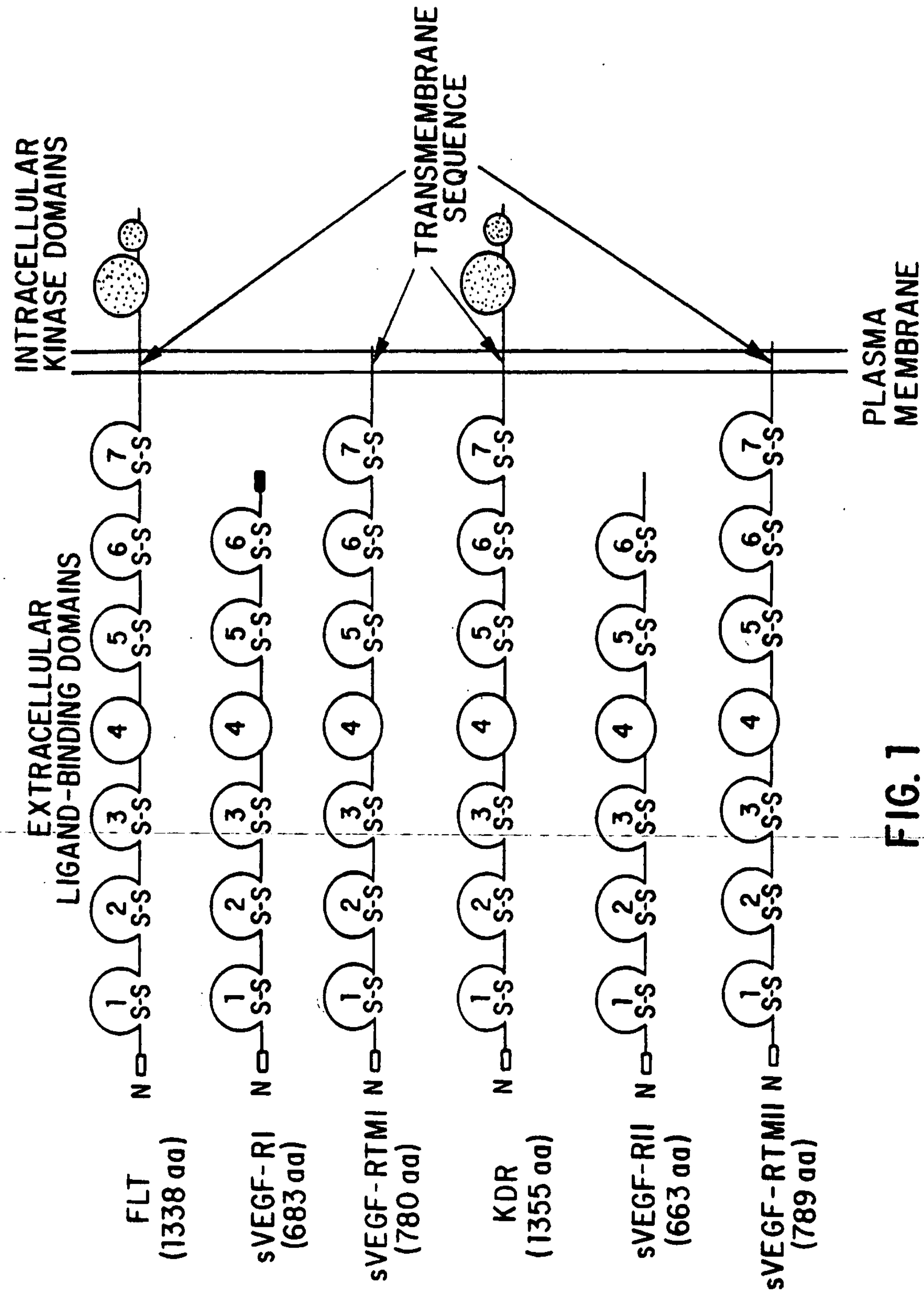

INTRACELLULAR KINASE DOMAINS
TRANSMEMBRANE SEQUENCE
PLASMA MEMBRANE
EXTRACELLULAR LIGAND-BINDING DOMAINS
FLT (1338 aa)
sVEGF-RI (683 aa)
sVEGF-RTMI (780 aa)
KDR (1355 aa)
sVEGF-RII (663 aa)
sVEGF-RTMII (789 aa)
N
1
2
3
4
5
6
7
S-S

FIG. 1

GCGGACACTCCTCTCGGCTCCTCCCCGGCAGCGGCGGCGGCTCGGAGCGGGCTCCGGGG
CTCGGGTGCAGCGGCCAGCGGGCCTGGCGGCGAGGATTACCCGGGGAAGTGGTTGTCTC
CTGGCTGGAGCCGCGAGACGGGCGCTCAGGGCGCGGGGCCGGCGGCGGCGAACGAGAG
GACGGACTCTGGCGGCCGGGTCGTTGGCCGGGGGAGCGCGGGCACCGGGCGAGCAGGC
CGCGTCGCGCTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTC
AGCTGTCTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGAGTTTA
AAAGGCACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGCAGGGGGGAAG
CAGCCCATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGAAAGGCTGAGCATAACT
AAATCTGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTACTTTAACCTTGAACACAGCTCAA
GCAAACCACACTGGCTTCTACAGCTGCAAATATCTAGCTGTACCTACTTCAAAGAAGAAGGA
AACAGAATCTGCAATCTATATATTTATTAGTGATACAGGTAGACCTTTCGTAGAGATGTACAG
TGAAATCCCCGAAATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTA
CGTCACCTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAA
AACGCATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATA
GGGCTTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACA
TCGACAAACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGTCAAATTACTTAG
AGGCCATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTCAAATGAC
CTGGAGTTACCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAAAGCA
ATTCCCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAAATGCAGAACAAAGACAAAG
GACTTTATACTTGTCGTGTAAGGAGTGGACCATCATTCAAATCTGTTAACACCTCAGTGCATA
TATATGATAAAGCATTCATCACTGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCT
GGCAAGCGGTCTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTAT

FIG. 2A

GGTTAAAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCG
TTAATTATCAAGGACGTAACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAA
CAGTCAAATGTGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTAC
GAAAAGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCC
TGACTTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCACCCCTGTAAC
CATAATCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAATGAAGAGTCCTTTATCCTGGAT
GCTGACAGCAACATGGGAAACAGAATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAG
GAAAGAATAAGATGGCTAGCACCTTGGTTGTGGCTGACTCTAGAATTTCTGGAATCTACATTT
GCATAGCTTCCAATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCACAGATGTG
CCAAATGGGTTTCATGTTAACTTGGAAAAAATGCCGACGGAAGGAGAGGACCTGAAACTGTC
TTGCACAGTTAACAAGTTCTTATACAGAGACGTTACTTGGATTTTACTGCGGACAGTTAATAA
CAGAACAATGCACTACAGTATTAGCAAGCAAAAAATGGCCATCACTAAGGAGCACTCCATCA
CTCTTAATCTTACCATCATGAATGTTTCCCTGCAAGATTCAGGCACCTATGCCTGCAGAGCCA
GGAATGTATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAATTACAATCAGAGGTGAGCAC
TGCAACAAAAAGGCTGTTTTCTCTCGGATCTCCAAATTTAAAAGCACAAGGAATGATTGTACC
ACACAAAGTAATGTAAAACATTAAAGGACTCATTAAAAAGTAACAGTTGTCTCATATCATCTTG
ATTTATTGTCACTGTTGCTAACTTTCAGGCTCGGAGGAGATGCTCCTCCCAAAATGAGTTCG
GAGATGATAGCAGTAATAATGAGACCCCCGGGCTCCAGCTCTGGGCCCCCCATTCAGGCCG
AGGGGGCTGCTCCGGGGGGCCGACTTGGTGCACGTTTGGATTTGGAGGATCCCTGCACTG
CCTTCTCTGTGTTTGTTGCTCTTGCTGTTTTCTCCTGCCTGATAAACAACAACTTGGGATGAT
CCTTTCCATTTTGATGCCAACCTCTTTTTATTTTTAAGCGGCGCCCTATAGT
(SEQ. ID. NO.: 5)

FIG. 2B

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQC
RGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYS
CKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP
NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYL
THRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKR
ASVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIY
DKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSAR
YLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFP
DPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARCDFCSNNEESFILD
ADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYICIASNKVGTVGRNISF
YITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMHYSIS
KQKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEEILQKKEITIRGEHCN
KKAVFSRISKFKSTRNDCTTQSNVKH (SEQ. ID. NO.: 6)

FIG. 3

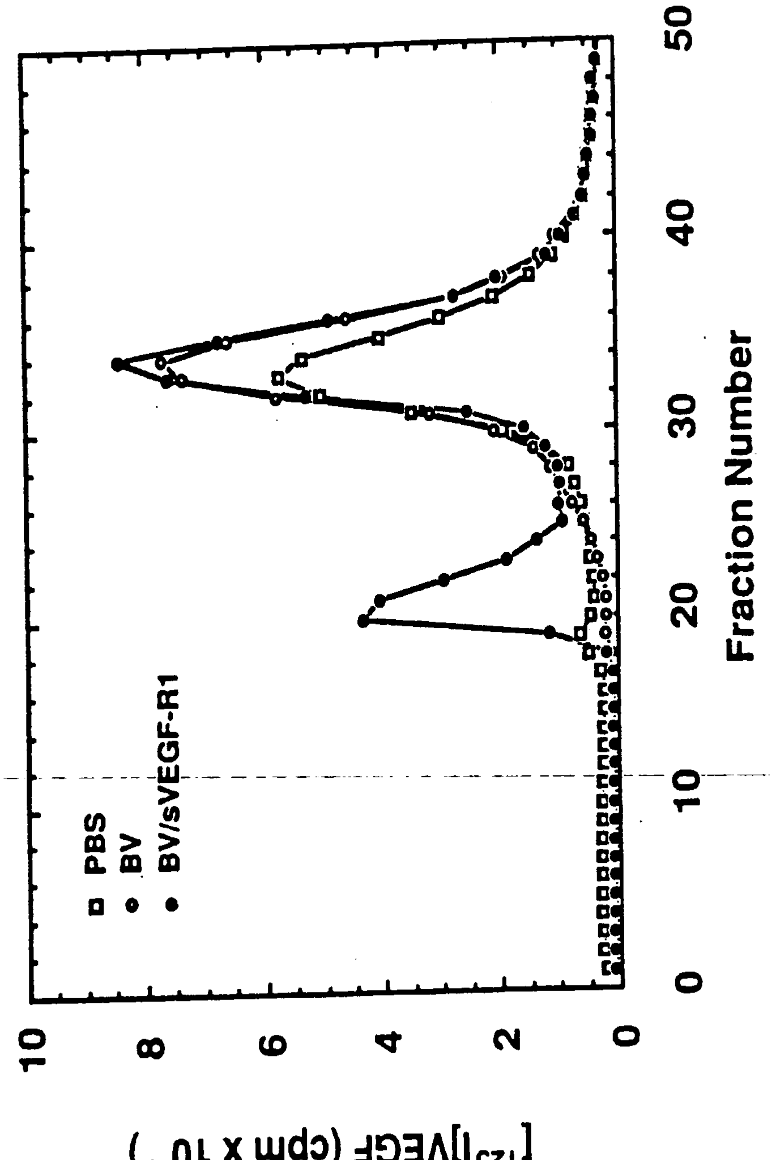

PBS
BV
BV/sVEGF-R1
Fraction Number
0
10
20
30
40
50
[125I]VEGF (cpm x 10-3)
0
2
4
6
8
10

FIG. 4

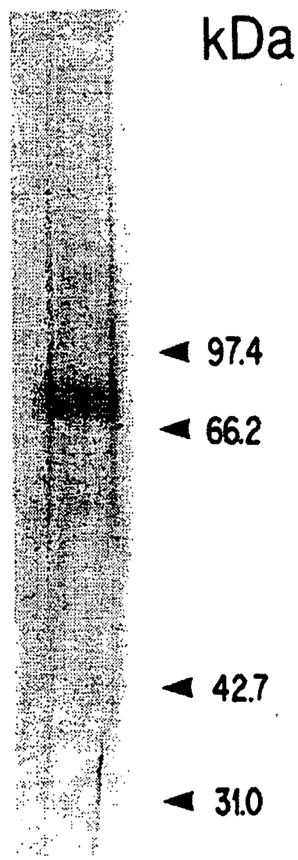


FIG. 5

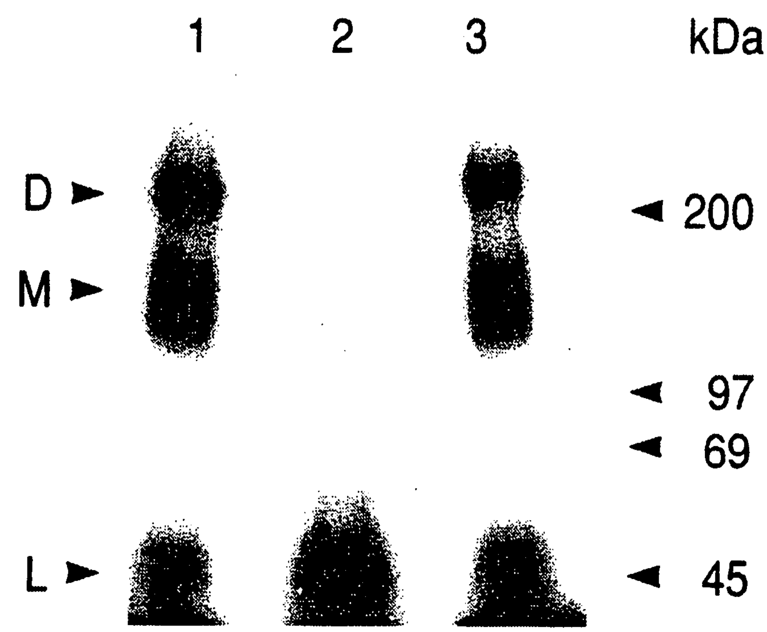


FIG. 6

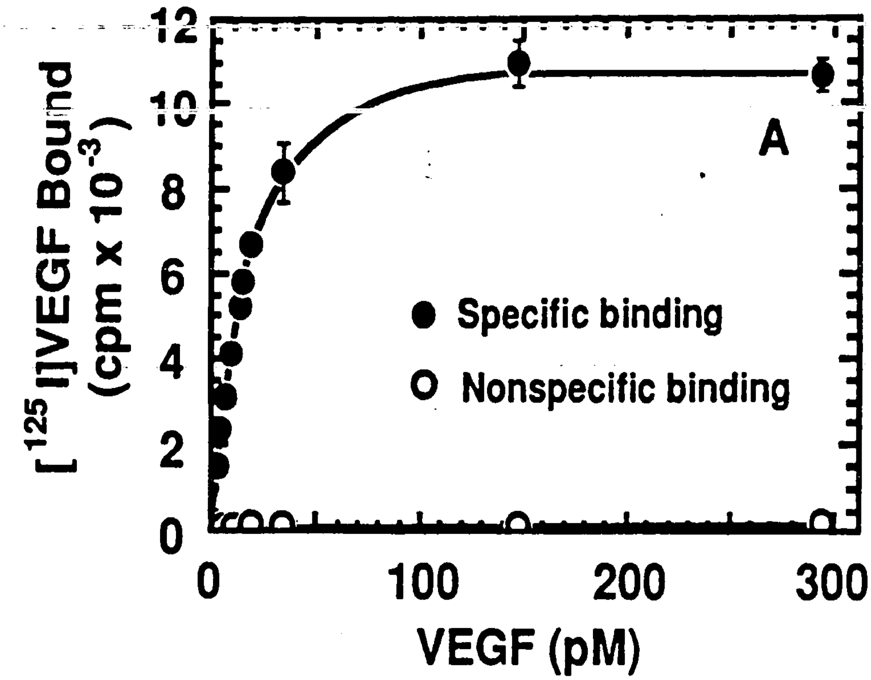

A
Specific binding
Nonspecific binding
[^{125}I]VEGF Bound (cpm x 10^{-3})
VEGF (pM)
0
2
4
6
8
10
12
0
100
200
300

FIG. 7A

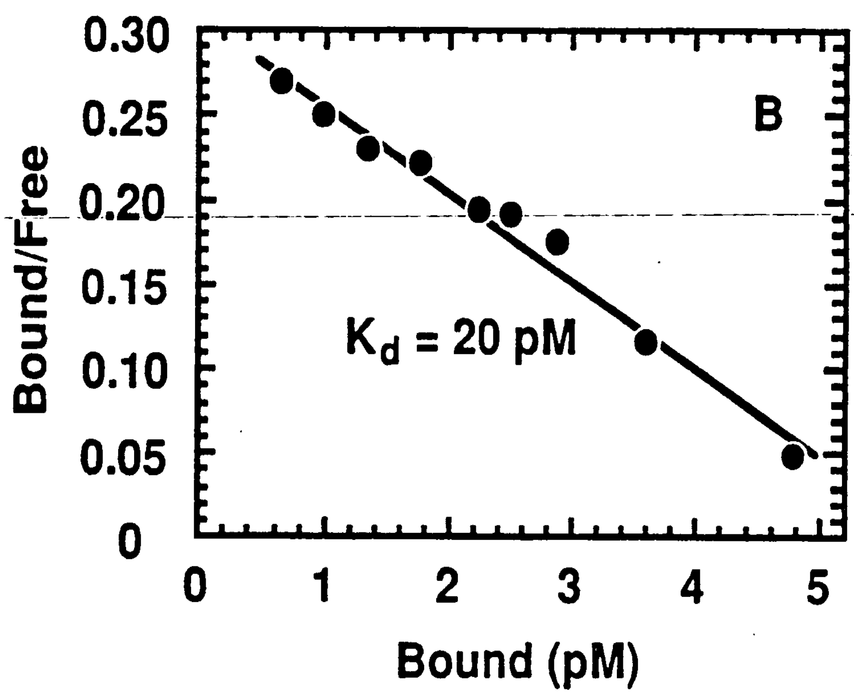
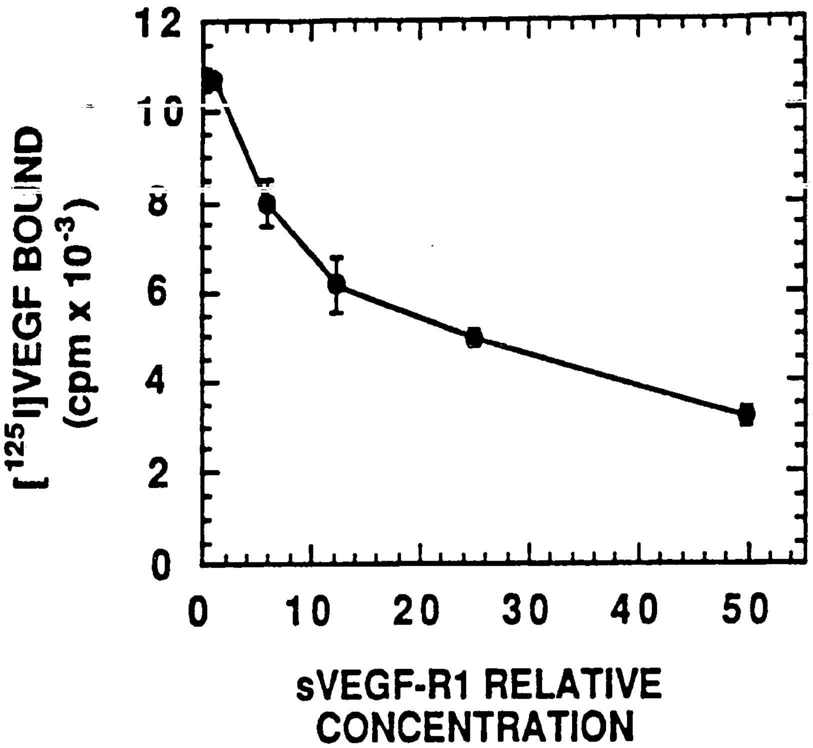
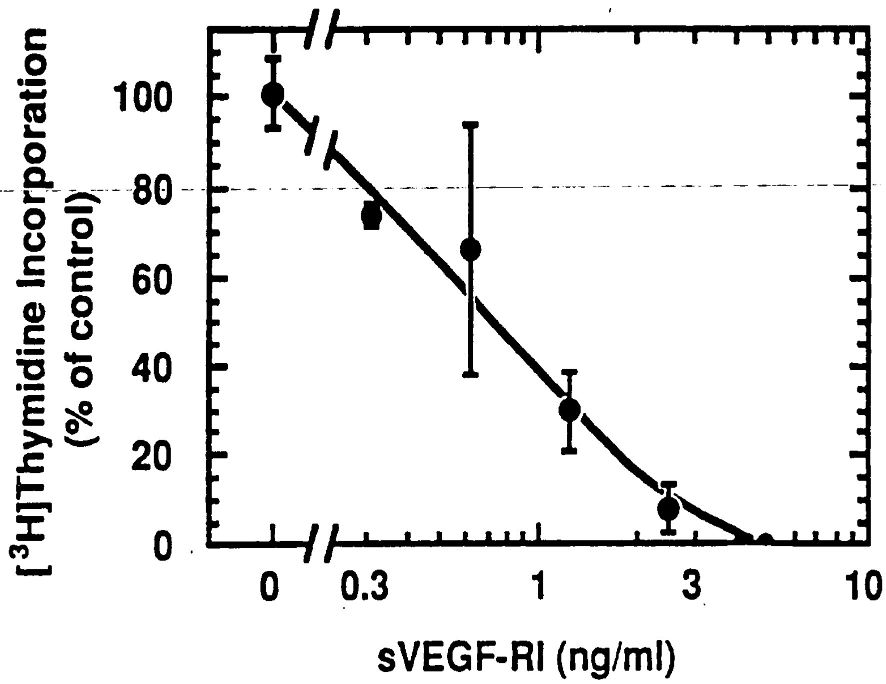

B
K_d = 20 pM
Bound/Free
Bound (pM)
0
0.05
0.10
0.15
0.20
0.25
0.30
0
1
2
3
4
5

FIG. 7B



12
10
8
6
4
2
0
[125I]VEGF BOUND
(cpm x 10-3)
0
10
20
30
40
50
sVEGF-R1 RELATIVE
CONCENTRATION

FIG. 8



100
80
60
40
20
0
[3H]Thymidine Incorporation
(% of control)
0
0.3
1
3
10
sVEGF-RI (ng/ml)

FIG. 9

GGTGTGGTCGCTGCGTTTCCTCTGCCTGCGCCGGGCATCACTTGCGCGCCGCAGAAAGTC
CGTCTGGCAGCCTGGATATCCTCTCCTACCGGCACCCGCAGACGCCCCTGCAGCCGCGGT
CGGCGCCCGGGCTCCCTAGCCCTGTGCGCTCAACTGTCCTGCGCTGCGGGGTGCCGCGAG
TTCCACCTCCGCGCCTCCTTCTCTAGACAGGCGCTGGGAGAAAGAACCGGCTCCCGAGTTC
CGGCATTTCGCCCGGCTCGAGGTGCAGGATGCAGAGCAAGGTGCTGCTGGCCGTCGCCCT
GTGGCTCTGCGTGGAGACCCGGGCCGCCTCTGTGGGTTTGCCTAGTGTTTCTCTTGATCTG
CCCAGGCTCAGCATACAAAAAGACATACTTACAATTAAGGCTAATACAACTCTTCAAATTACT
TGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAGCAAA
GGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCACAATTCCAAAAGT
GATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAAACTGACTTGGCCTCGGTC
ATTTATGTCTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAG
TCGTGTACATTACTGAGAACAAAAACAAAACTGTGGTGATTCCATGTCTCGGGTCCATTTCAA
ATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAAGAGATTTGTTCCTGATGGTAACAGAA
TTTCCTGGGACAGCAAGAAGGGCTTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATG
GTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTACCAGTCTATTATGTACATAGTTGTCGTT
GTAGGGTATAGGATTTATGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGA
GAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGG
GAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCAGTCT
GGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGTGACCA

FIG. 10A

AGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTTGTCA
GGGTCCATGAAAAACCTTTTGTTGCTTTTGGAAGTGGCATGGAATCTCTGGTGGAAGCCACG
GTGGGGGAGCGTGTCAGAATCCCTGCGAAGTACCTTGGTTACCCACCCCCAGAAATAAAAT
GGTATAAAAATGGAATACCCCTTGAGTCCAATCACACAATTAAAGCGGGGCATGTACTGACG
ATTATGGAAGTGAGTGAAAGAGACACAGGAAATTACACTGTCATCCTTACCAATCCCATTTCA
AAGGAGAAGCAGAGCCATGTGGTCTCTCTGGTTGTGTATGTCCCACCCCAGATTGGTGAGA
AATCTCTAATCTCTCCTGTGGATTCCTACCAGTACGGCACCACTCAAACGCTGACATGTACG
GTCTATGCCATTCCTCCCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGCG
CCAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCCTTGTGAAGAATGGAGAAG
TGTGGAGGACTTCCAGGGAGGAAATAAAATTGCCGTTAATAAAAATCAATTTGCTCTAATTGA
AGGAAAAAACAAAACTGTAAGTACCCTTGTTATCCAAGCGGCAAATGTGTCAGCTTTGTACAA
ATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTGATCTCCTTCCACGTGACCAGG
GGTCCTGAAATTACTTTGCAACCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTG
GTGCACTGCAGACAGATCTACGTTTGAGAACCTCACATGGTACAAGCTTGGCCCACAGCCTC
TGCCAATCCATGTGGGAGAGTTGCCCACACCTGTTTGCAAGAACTTGGATACTCTTTGGAAA
TTGAATGCCACCATGTTCTCTAATAGCACAAATGACATTTTGATCATGGAGCTTAAGAATGCA
TCCTTGCAGGACCAAGGAGACTATGTCTGCCTTGCTCAAGACAGGAAGACCAAGAAAAGAC
ATTGCGTGGTCAGGCAGCTCACAGTCCTAGAGCGTTAA (SEQ. ID. NO.: 16)

FIG. 10B

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQ
RDLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETD
LASVIYVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARY
PEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVG
YRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVN
RDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK
PFVAFGSGMESLVEATVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHV
LTIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYG
TTQTLTCTVYAIPPPHHIHWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDF
QGGNKIAVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFH
VTRGPEITLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQPLPIHVGELPT
PVCKNLDTLWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRH
CVVRQLTVLER··· (SEQ. ID. NO.: 13)

FIG. 11

GGTGTGGTCGCTGCGTTTCCTCTGCCTGCGCCGGGCATCACTTGCGCGCCGCAGAAAGTC
CGTCTGGCAGCCTGGATATCCTCTCCTACCGGCACCCGCAGACGCCCCTGCAGCCGCGGT
CGGCGCCCGGGCTCCCTAGCCCTGTGCGCTCAACTGTCCTGCGCTGCGGGGTGCCGCGAG
TTCCACCTCCGCGCCTCCTTCTCTAGACAGGCGCTGGGAGAAAGAACCGGCTCCCGAGTTC
CGGCATTTCGCCCGGCTCGAGGTGCAGGATGCAGAGCAAGGTGCTGCTGGCCGTCGCCCT
GTGGCTCTGCGTGGAGACCCGGGCCGCCTCTGTGGGTTTGCCTAGTGTTTCTCTTGATCTG
CCCAGGCTCAGCATACAAAAAGACATACTTACAATTAAGGCTAATACAACTCTTCAAATTACT
TGCAGGGGACAGAGGGACTTGGACTGGCTTTGGCCCAATAATCAGAGTGGCAGTGAGCAAA
GGGTGGAGGTGACTGAGTGCAGCGATGGCCTCTTCTGTAAGACACTCACAATTCCAAAAGT
GATCGGAAATGACACTGGAGCCTACAAGTGCTTCTACCGGGAAACTGACTTGGCCTCGGTC
ATTTATGTCTATGTTCAAGATTACAGATCTCCATTTATTGCTTCTGTTAGTGACCAACATGGAG
TCGTGTACATTACTGAGAACAAAAACAAAACTGTGGTGATTCCATGTCTCGGGTCCATTTCAA
ATCTCAACGTGTCACTTTGTGCAAGATACCCAGAAAAGAGATTTGTTCCTGATGGTAACAGAA
TTTCCTGGGACAGCAAGAAGGGCTTTACTATTCCCAGCTACATGATCAGCTATGCTGGCATG
GTCTTCTGTGAAGCAAAAATTAATGATGAAAGTTACCAGTCTATTATGTACATAGTTGTCGTT
GTAGGGTATAGGATTTATGATGTGGTTCTGAGTCCGTCTCATGGAATTGAACTATCTGTTGGA
GAAAAGCTTGTCTTAAATTGTACAGCAAGAACTGAACTAAATGTGGGGATTGACTTCAACTGG
GAATACCCTTCTTCGAAGCATCAGCATAAGAAACTTGTAAACCGAGACCTAAAAACCCAGTCT
GGGAGTGAGATGAAGAAATTTTTGAGCACCTTAACTATAGATGGTGTAACCCGGAGTGACCA
AGGATTGTACACCTGTGCAGCATCCAGTGGGCTGATGACCAAGAAGAACAGCACATTTGTCA
GGGTCCATGAAAAACCTTTTGTTGCTTTTGGAAGTGGCATGGAATCTCTGGTGGAAGCCACG
GTGGGGGAGCGTGTCAGAATCCCTGCGAAGTACCTTGGTTACCCACCCCCAGAAATAAAAT

FIG. 12A

GGTATAAAAATGGAATACCCCTTGAGTCCAATCACACAATTAAAGCGGGGCATGTACTGACG
ATTATGGAAGTGAGTGAAAGAGACACAGGAAATTACACTGTCATCCTTACCAATCCCATTTCA
AAGGAGAAGCAGAGCCATGTGGTCTCTCTGGTTGTGTATGTCCCACCCCAGATTGGTGAGA
AATCTCTAATCTCTCCTGTGGATTCCTACCAGTACGGCACCACTCAAACGCTGACATGTACG
GTCTATGCCATTCCTCCCCCGCATCACATCCACTGGTATTGGCAGTTGGAGGAAGAGTGCG
CCAACGAGCCCAGCCAAGCTGTCTCAGTGACAAACCCATACCCTTGTGAAGAATGGAGAAG
TGTGGAGGACTTCCAGGGAGGAAATAAAATTGCCGTTAATAAAAATCAATTTGCTCTAATTGA
AGGAAAAAACAAAACTGTAAGTACCCTTGTTATCCAAGCGGCAAATGTGTCAGCTTTGTACAA
ATGTGAAGCGGTCAACAAAGTCGGGAGAGGAGAGAGGGTGATCTCCTTCCACGTGACCAGG
GGTCCTGAAATTACTTTGCAACCTGACATGCAGCCCACTGAGCAGGAGAGCGTGTCTTTGTG
GTGCACTGCAGACAGATCTACGTTTGAGAACCTCACATGGTACAAGCTTGGCCCACAGCCTC
TGCCAATCCATGTGGGAGAGTTGCCCACACCTGTTTGCAAGAACTTGGATACTCTTTGGAAA
TTGAATGCCACCATGTTCTCTAATAGCACAAATGACATTTTGATCATGGAGCTTAAGAATGCA
TCCTTGCAGGACCAAGGAGACTATGTCTGCCTTGCTCAAGACAGGAAGACCAAGAAAAGAC
ATTGCGTGGTCAGGCAGCTCACAGTCCTAGAGCGTGTGGCACCCACGATCACAGGAAACCT
GGAGAATCAGACGACAAGTATTGGGGAAAGCATCGAAGTCTCATGCACGGCATCTGGGAAT
CCCCCTCCACAGATCATGTGGTTTAAAGATAATGAGACCCTTGTAGAAGACTCAGGCATTGT
ATTGAAGGATGGGAACCGGAACCTCACTATCCGCAGAGTGAGGAAGGAGGACGAAGGCCT
CTACACCTGCCAGGCATGCAGTGTTCTTGGCTGTGCAAAAGTGGAGGCATTTTTCATAATAG
AAGGTGCCCAGGAAAAGACGAACTTGGAAATCATTATTCTAGTAGGCACGACGGTGATTGCC
ATGTTCTTCTGGCTACTTCTTGTCATCATCCTAGGGACCGTTTAA (SEQ. ID. NO.: 18)

FIG. 12B

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQ
RDLDWLWPNNQSGSEQRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETD
LASVIYVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARY
PEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVG
YRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVN
RDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEK
PFVAFGSGMESLVEATVGERVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHV
LTIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPQIGEKSLISPVDSYQYG
TTQTLTCTVYAIPPPHHIHWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDF
QGGNKIAVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFH
VTRGPEITLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQPLPIHVGELPT
PVCKNLDTLWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRH
CVVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLV
EDSGIVLKDGNRNLTIRRVRKEDEGLYTCQACSVLGCAKVEAFFIIEGAQEKTNL
EIIILVGTTVIAMFFWLLLVIILGTV··· (SEQ. ID. NO.: 15)

FIG. 13

GCGCTCACCATGGTCAGCTACTGGGACACCGGGGTCCTGCTGTGCGCGCTGCTCAGCTGT
CTGCTTCTCACAGGATCTAGTTCAGGTTCAAAATTAAAAGATCCTGAACTGAGTTTAAAAGGC
ACCCAGCACATCATGCAAGCAGGCCAGACACTGCATCTCCAATGCAGGGGGGAAGCAGCC
CATAAATGGTCTTTGCCTGAAATGGTGAGTAAGGAAAGCGAAAGGCTGAGCATAACTAAATC
TGCCTGTGGAAGAAATGGCAAACAATTCTGCAGTACTTTAACCTTGAACACAGCTCAAGCAA
ACCACACTGGCTTCTACAGCTGCAAATATCTAGCTGTACCTACTTCAAAGAAGAAGGAAACA
GAATCTGCAATCTATATATTTATTAGTGATACAGGTAGACCTTTCGTAGAGATGTACAGTGAA
ATCCCCGAAATTATACACATGACTGAAGGAAGGGAGCTCGTCATTCCCTGCCGGGTTACGTC
ACCTAACATCACTGTTACTTTAAAAAAGTTTCCACTTGACACTTTGATCCCTGATGGAAAACG
CATAATCTGGGACAGTAGAAAGGGCTTCATCATATCAAATGCAACGTACAAAGAAATAGGGC
TTCTGACCTGTGAAGCAACAGTCAATGGGCATTTGTATAAGACAAACTATCTCACACATCGAC
AAACCAATACAATCATAGATGTCCAAATAAGCACACCACGCCCAGTCAAATTACTTAGAGGC
CATACTCTTGTCCTCAATTGTACTGCTACCACTCCCTTGAACACGAGAGTTCAAATGACCTGG
AGTTACCCTGATGAAAAAAATAAGAGAGCTTCCGTAAGGCGACGAATTGACCAAAGCAATTC
CCATGCCAACATATTCTACAGTGTTCTTACTATTGACAAAATGCAGAACAAAGACAAAGGACT
TTATACTTGTCGTGTAAGGAGTGGACCATCATTCAAATCTGTTAACACCTCAGTGCATATATA
TGATAAAGCATTCATCACTGTGAAACATCGAAAACAGCAGGTGCTTGAAACCGTAGCTGGCA
AGCGGTCTTACCGGCTCTCTATGAAAGTGAAGGCATTTCCCTCGCCGGAAGTTGTATGGTTA
AAAGATGGGTTACCTGCGACTGAGAAATCTGCTCGCTATTTGACTCGTGGCTACTCGTTAAT

FIG. 14A

TATCAAGGACGTAACTGAAGAGGATGCAGGGAATTATACAATCTTGCTGAGCATAAAACAGT
CAAATGTGTTTAAAAACCTCACTGCCACTCTAATTGTCAATGTGAAACCCCAGATTTACGAAA
AGGCCGTGTCATCGTTTCCAGACCCGGCTCTCTACCCACTGGGCAGCAGACAAATCCTGAC
TTGTACCGCATATGGTATCCCTCAACCTACAATCAAGTGGTTCTGGCACCCCCTGTAACCATAA
TCATTCCGAAGCAAGGTGTGACTTTTGTTCCAATAATGAAGAGTCCTTTATCCTGGATGCTGA
CAGCAACATGGGAAACAGAATTGAGAGCATCACTCAGCGCATGGCAATAATAGAAGGAAAG
AATAAGATGGCTAGCACCTTGGTTGTGGCTGACTCTAGAATTTCTGGAATCTACATTTGCATA
GCTTCCAATAAAGTTGGGACTGTGGGAAGAAACATAAGCTTTTATATCACAGATGTGCCAAAT
GGGTTTCATGTTAACTTGGAAAAAATGCCGACGGAAGGAGAGGACCTGAAACTGTCTTGCAC
AGTTAACAAGTTCTTATACAGAGACGTTACTTGGATTTTACTGCGGACAGTTAATAACAGAAC
AATGCACTACAGTATTAGCAAGCAAAAAATGGCCATCACTAAGGAGCACTCCATCACTCTTAA
TCTTACCATCATGAATGTTTCCCTGCAAGATTCAGGCACCTATGCCTGCAGAGCCAGGAATG
TATACACAGGGGAAGAAATCCTCCAGAAGAAAGAAATTACAATCAGAGATCAGGAAGCACCA
TACCTCCTGCGAAACCTCAGTGATCACACAGTGGCCATCAGCAGTTCCACCACTTTAGACTG
TCATGCTAATGGTGTCCCCGAGCCTCAGATCACTTGGTTTAAAAACAACCACAAAATACAACA
AGAGCCTGGAATTATTTTAGGACCAGGAAGCAGCACGCTGTTTATTGAAAGAGTCACAGAAG
AGGATGAAGGTGTCTATCACTGCAAAGCCACCAACCAGAAGGGCTCTGTGGAAAGTTCAGC
ATACCTCACTGTTCAAGGAACCTCGGACAAGTCTAATCTGGAGCTGATCACTCTAACATGCA
CCTGTGTGGCTGCGACTCTCTTCTGGCTCCTATTAACCCTCCTTATCTAA (SEQ. ID. NO.: 17)

FIG. 14B

MVSYWDTGVLLCALLSCLLLTGSSSGSKLKDPELSLKGTQHIMQAGQTLHLQC
RGEAAHKWSLPEMVSKESERLSITKSACGRNGKQFCSTLTLNTAQANHTGFYS
CKYLAVPTSKKKETESAIYIFISDTGRPFVEMYSEIPEIIHMTEGRELVIPCRVTSP
NITVTLKKFPLDTLIPDGKRIIWDSRKGFIISNATYKEIGLLTCEATVNGHLYKTNYL
THRQTNTIIDVQISTPRPVKLLRGHTLVLNCTATTPLNTRVQMTWSYPDEKNKR
ASVRRRIDQSNSHANIFYSVLTIDKMQNKDKGLYTCRVRSGPSFKSVNTSVHIY
DKAFITVKHRKQQVLETVAGKRSYRLSMKVKAFPSPEVVWLKDGLPATEKSAR
YLTRGYSLIIKDVTEEDAGNYTILLSIKQSNVFKNLTATLIVNVKPQIYEKAVSSFP
DPALYPLGSRQILTCTAYGIPQPTIKWFWHPCNHNHSEARCDFCSNNEESFILD
ADSNMGNRIESITQRMAIIEGKNKMASTLVVADSRISGIYICIASNKVGTVGRNISF
YITDVPNGFHVNLEKMPTEGEDLKLSCTVNKFLYRDVTWILLRTVNNRTMHYSIS
KQKMAITKEHSITLNLTIMNVSLQDSGTYACRARNVYTGEEILQKKEITIRDQEAP
YLLRNLSDHTVAISSSTTLDCHANGVPEPQITWFKNNHKIQQEPGIILGPGSSTLF
IERVTEEDEGVYHCKATNQKGSVESSAYLTVQGTSDKSNLELITLTCTCVAATLF
WLLLTLLI (SEQ. ID. NO.:14)

FIG. 15

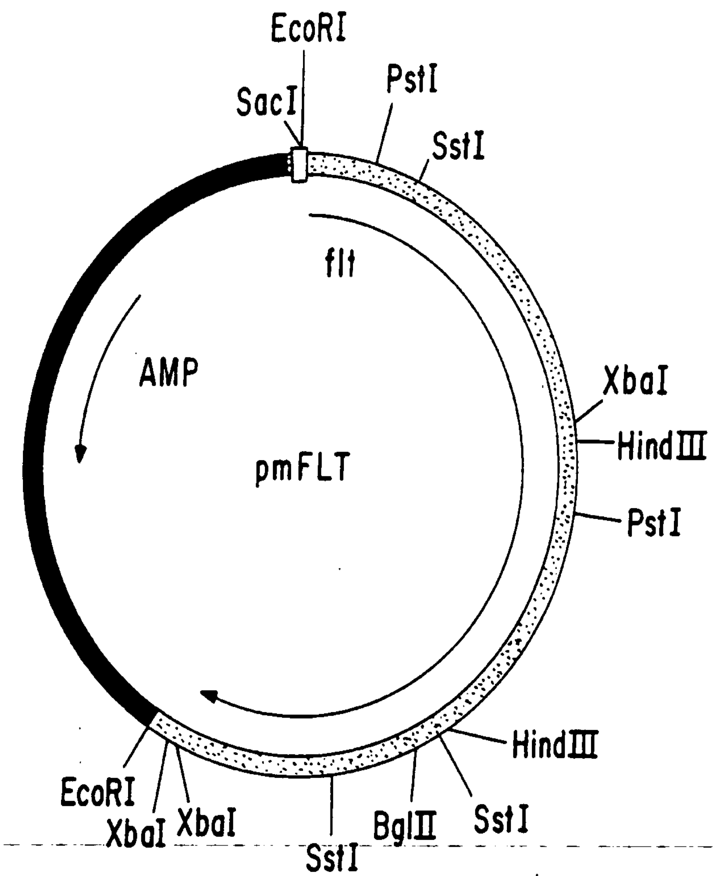

EcoRI
SacI
PstI
SstI
flt
AMP
pmFLT
XbaI
HindIII
PstI
HindIII
EcoRI
XbaI
XbaI
SstI
BglII
SstI

FIG. 16

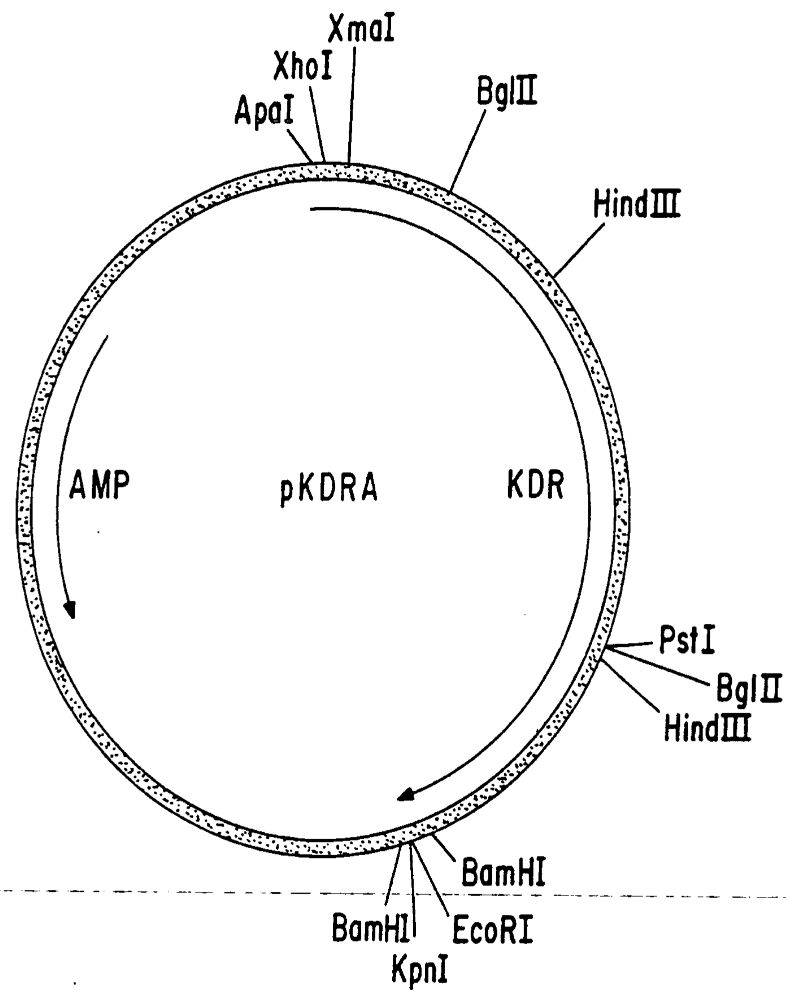

XmaI
XhoI
ApaI
BglII
HindIII
AMP
pKDRA
KDR
PstI
BglII
HindIII
BamHI
BamHI
EcoRI
KpnI

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01957

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C07K 13/00; C12P 21/00; C12N 5/00, 15/00
US CL :435/69.1, 240.1, 320.1; 530/350; 536/23.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 240.1, 320.1; 530/350; 536/23.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Medline, Biosis, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	Journal of Cellular Physiology, Volume 149, Number 1, issued October 1991, Bikfalvi et al, "Interaction of Vasculotropin/Vascular Endothelial Cell Growth Factor with Human Umbilical Vein Endothelial Cells: Binding, Internalization, Degradation, and Biological Effects", pages 50-59, see abstract.	1 ----- 14, 15, 18
Y	Science, Volume 255, issued 21 February 1992, De Vries et al, "The fms-Like Tyrosine Kinase, a Receptor for Vascular endothelial Growth Factor", pages 989-991, see abstract and fig. 1.	1-18

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 MAY 1994	JUN 03 1994

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Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Sally P. Teng Jill Warden for Telephone No. (703) 308-0196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Oncogene, Volume 5, issued 1990, Shibuya et al, "Nucleotide Seqence and Expression of a Novel Human Receptor-Type Tyrosine Kinase Gene (flt) Closely Related to the fms Family", pages 519-524, see abstract and page 521.	1-18
Y	Biochemical and Biophysical Research Communications, Volume 187, Number 3, issued 30 September 1992, Terman et al, "Identification of the KDR Tyrosine Kinase as a Receptor for Vascular Endothelial Cell Growth Factor", pages 1579-1586, see summary and page 1583.	1-18